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IMPROVED PHOSPHORUS AVAILABILITY IN POULTRY FED WHEAT/CANOLA MEAL-BASED DIETS SUPPLEMENTED WITH PHYTASE ENZYME

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Lesley William John Nernberg

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

Department of Animal Science

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ABSTRACT

A series of experiments were conducted to examine the effect of phytase supplementation on phytate digestibility and P utilization of wheat/canola meal diets fed to young broiler chickens. An *in vitro* procedure was used to determine the rate of phytate hydrolysis under simulated conditions of the gastrointestinal tract. Complete hydrolysis of phytate in canola meal by phytase was accomplished under conditions studied. Addition of calcium carbonate to canola meal linearly decreased degree of phytate hydrolysis regardless of level of phytase supplementation. Phytate hydrolysis in wheat was dependent on the degree of grinding with 37 and 61% of phytate hydrolyzed, respectively, in wheat ground to pass through a 5 mm and 1 mm sieve. Only partial hydrolysis (46%) of phytate was attained *in vitro* when a wheat/canola meal diet containing phytase enzyme was used.

Two *in vivo* studies were conducted to determine the effect of phytase on phytate P utilization in broiler chickens fed low phosphorus wheat/canola meal diets. In the first trial, broiler chickens were fed diets containing two levels of non-phytate P (nP), 0.25 and 0.35%, each supplemented with phytase enzyme to provide 0, 500, and 1000 units/kg diet. A diet containing 0.45% nP served as a positive control. In comparison to the positive control, no differences in feed intake, weight gain, and feed to gain ratio were observed for the 0.35% nP diet regardless of phytase supplementation. The addition of 500 and 1000 units phytase/kg to 0.25% nP diets linearly improved ($P<0.05$) feed intake and weight gain. Phytase supplementation increased ($P<0.05$) phytate digestibility, toe ash percentage, and reduced P excretion by 37%. A second *in vivo* study was conducted to examine the effect

of Ca, cholecalciferol, and phytase on phytate P utilization. A low nP wheat/canola meal diet was supplemented with two levels of Ca (0.7 or 0.8%), cholecalciferol (1000 or 5000 IU), and phytase (0 or 1000 units/kg). In comparison to the positive control, no differences in feed intake, weight gain, and feed to gain ratio were observed for the 0.7% Ca diets regardless of phytase supplementation. The addition of phytase improved ($P<0.05$) feed intake, body weight gain, phytate and Ca digestibility, and toe ash percentage of broilers fed low nP diets containing 0.8% Ca. The present studies suggest that phytase supplementation of low nP wheat/canola meal diets improves phytate P utilization, reduces P excretion, and demonstrate that level of Ca in the diet is a critical factor influencing phytate digestibility.

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AA	Amino acid
DM	Dry matter
nP	Non-phytate phosphorus
PA	Phytic acid
SBM	Soybean meal
tP	Total phosphorus
U	Units
WC	Wheat ground coarse
WF	Wheat ground fine

CHAPTER 1

INTRODUCTION

Although natural feed ingredients contain reasonable quantities of phosphorus (P), generally, the availability of P in monogastric (pig and poultry) diets is quite low. Monogastric diets consist largely of seeds (cereal grains) and seed by-products (oilseed meals) where the majority of P is bound as an organic complex called phytic acid or its salt, phytate. Phosphorus in this form is poorly utilized by pigs and poultry because they lack sufficient phytase, the enzyme required to cleave the P from the phytate molecule. The inability or inadequacy of monogastrics to efficiently utilize phytate P presents several problems. First, since only a small portion of the P from plant derived ingredients is utilized, inorganic phosphate must be supplemented to the diet in order to meet the animal's requirement. Among the mineral supplements inorganic phosphorus sources are the most expensive. Secondly, a large amount of P excreted in the manure poses an environmental concern, especially in areas of intensive animal production (eg. Netherlands, Fraser River Valley). Excess phosphorus in manure can move into ground and fresh water sources where it stimulates the growth of algae (ie. algal blooms) and other aquatic plants (Sharpley and Menzel, 1987). This process, called "eutrophication" results in a marked deterioration in the quality of fresh water. Phosphorus and nitrogen are currently the two elements in manure that are of greatest concern and limit the rate of land application. (Kornegay, 1996). Several European countries have implemented strict legislation to control pollution caused by animal manure. These regulations may be enacted in certain parts of the USA and Canada in the near

future. Another nutritional concern associated with phytate is its ability to complex with minerals and protein. Phytic acid has been shown to significantly reduce the absorption and availabilty of several trace elements and amino acids (Torre et al., 1991). The hydrolysis of the phytate complex may liberate minerals and proteins making them more available to the animal.

The addition of phytase enzyme to pig and poultry diets may be an effective strategy to overcome the problems associated with phytate and its poor availability. The use of microbial phytase in poultry diets to improve P availability has long been established. Nelson et al. (1971) used a microbial phytase produced by *Aspergillus niger* that improved the availability of P in corn and soybean meal (SBM) for chicks. However, in the past, low supply and high cost of microbial phytase limited its commercial use. More recently, the production of microbial phytase has become more economical and improved through advanced biotechnology and increased concern for the environment. This has led to a renewed interest in the use of dietary phytase enzyme in poultry diets. It has been reported that microbial phytase added to low P corn/SBM diets increased P availability in broiler chickens to over 60% and decreased the amount of P excreted by 30-50% (Simons et al., 1990; Perney et al., 1993; Broz et al., 1994; Kornegay et al., 1996; Yi et al., 1996c).

Much of the work with phytase has been done with corn/SBM diets. In Western Canada, however, wheat and canola meal are common feed ingredients. Canola meal, a by-product of the canola crushing industry, is a good source of crude protein (36%, NRC 1994) and provides a reasonable source of amino acids for poultry feeds. It also contains a high level of phytate (2.9%) compared to other feed ingredients, thus, the addition of phytase

enzyme to canola meal containing diets may greatly improve the nutritive value of this feedstuff. Wheat is an excellent energy source in diets and also contains a high amount of endogenous plant phytase. Thus, the use of wheat along with exogenous phytase supplementation in poultry diets may aid in improving P availability.

The major objective of this study was to determine the effect of a commercial phytase enzyme preparation on the phytate hydrolysis of a wheat/canola meal diet and the subsequent improvement in P availability. This was accomplished through the use of an *in vitro* procedure which simulated the gastrointestinal tract conditions of the chicken. A broiler performance study was used to substantiate the *in vitro* results. A secondary objective was to investigate the factors which affect the extent of phytate hydrolysis in wheat/canola meal-based diets using both *in vitro* and *in vivo* experimentation.

CHAPTER 2

REVIEW OF THE LITERATURE

Structure and chemistry of phytic acid

The phytic acid (PA) molecule consists of an inositol ring structure with six phosphate groups attached. The generally accepted structure of phytic acid, or myoinositol 1,2,3,4,5,6-hexakis-dihydrogen phosphate is shown in Figure 1. Phytate and phytin are other terms associated with phytic acid which are prevalently used in the literature. Phytin implies a calcium-magnesium salt of phytic acid, whereas phytate is the mono to dodeca anion of phytic acid (Maga, 1982). There are 12 replaceable protons in the phytic acid molecule. Costello et al. (1976) using ^{31}P nuclear magnetic resonance-pH titration methods found that six are in the strong acid range (pKa 1.5), one in the weak acid range (pKa 5.7), two with pKa 6.8 to 7.6, and three very weak acids (pKa >10). This suggests that at all pH values normally encountered in feedstuffs, phytic acid will be negatively charged. This makes phytic acid a strong chelating compound which has the ability to bind cations and/or protein.

Physiological function of phytic acid in plants

Phytic acid, or phytate, is the chief storage form of phosphorus in seeds. In mature seeds, phytic acid occurs primarily as a complex salt of mono- and divalent cations and/or protein (Lott, 1984). It accumulates in seeds during the ripening period and serves several important physiological functions during dormancy and germination. These include

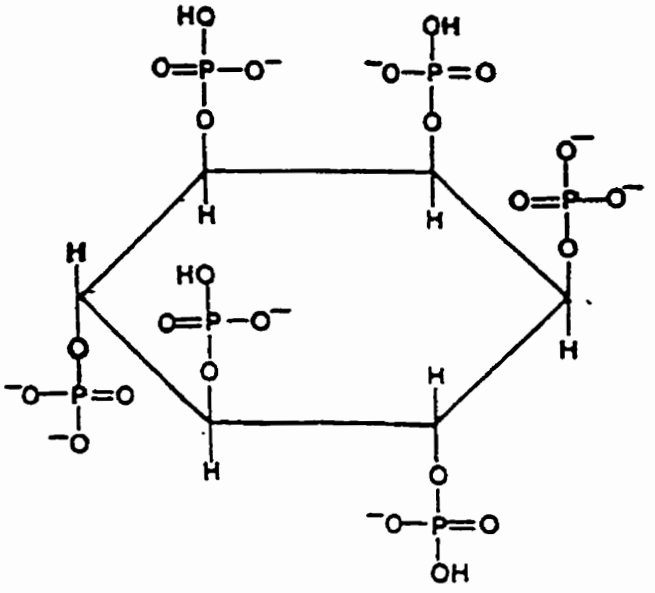


FIGURE 1. Structure of phytic acid (From Cheryan, 1980).

initiation of dormancy, antioxidant protection during dormancy (Graf et al., 1987), and storage of phosphorus, high energy phosphoryl groups, and cations for use during germination (Williams, 1970).

Occurrence of phytate

Phytic acid occurs in a wide variety of plant materials with the greatest concentration being found in mature seeds. The phytate P content of some common feed ingredients are presented in Table 1. Phytate P constitutes 60 to 80% of the total P in cereals and oilseed meals. The amount of phytate P varies from 0.21% in oats and corn to 0.97% in wheat bran. The concentration of phytate P depends on the part of the plant from which feedstuffs are derived. Cereal grains contain moderate amounts whereas oilseed meals and cereal by-products contain large amounts.

The phytate content of seeds may vary depending on climate conditions, location, irrigation, type of soil, fertilizer application, and year during which a cultivar or variety is grown (Reddy et al., 1989). Nahapetian and Bassiri (1976) reported variations in the phytate content of wheat grown in different years. The authors attributed the changes in concentration of phytate to environmental factors such as rainfall and temperature in a given year. Nitrogen and phosphorus fertilization was found to affect the phytic acid content of oats (Saastamoinen, 1987). In contrast, Barrier-Guillot et al. (1996) showed that N and P fertilization, date of harvest, and variety did not significantly affect the phytate P content of wheat.

Phytic acid accumulates in specific morphological components in the seed. In wheat,

TABLE 1. Phytate P content of some common feed ingredients

Ingredient	Phytate P (%)	Phytate P, as % total P	Reference
Corn	0.21	72	*
Barley	0.22	60	**
Wheat	0.29	71	*
Oats	0.21	58	**
Rye	0.22	61	**
Wheat bran	0.97	84	**
Canola meal	0.78	70	*
Soybean meal	0.39	57	*

* Kirby and Nelson, 1988

** Eeckhout and De Paepe, 1994

85% of the phytate is located in the aleurone layer, 13% in the germ and 2% in the endosperm (O'Dell et al., 1972). Eighty-eight percent of the phytate in corn is located in the germ portion of the kernel. In oilseeds such as canola, phytate is found in the crystalline globoids inside protein bodies in the cell of the radicle and primarily the cotyledon (Yiu et al., 1982). However, phytates in soybean are unique in that, although associated with globoids, they appear to have no specific site of localization (Prattley and Stanley, 1982).

Phytic acid-mineral interactions

As mentioned previously, phytic acid has strong chelating potential due to the multiplicity of negatively charged phosphate groups. It may bind di- and trivalent cations such as Zn, Ca, Mg, Fe, and Cu to form insoluble salts potentially rendering these minerals unavailable for intestinal absorption. Phytic acid can complex a cation within a phosphate group itself, between two phosphate groups of a molecule, or between groups of different phytic acid molecules (Cheryan, 1980).

Phytate will form stable complexes with minerals of varying affinities depending upon the metal ions involved. Maddaiah et al. (1964b) found that zinc has the highest affinity for phytic acid at pH 7.4, followed by Cu^{2+}, Co^{2+}, Mn^{2+}, and Ca^{2+}. Vohra et al. (1965) reported phytate complexes with minerals in the following descending order: Cu^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Fe^{3+}, Ca^{2+}. The previous studies suggest that phytate-mineral complexes may precipitate at intestinal pH. Nutritional deficiencies are thought to occur due to the insolubility of metal salts of phytic acid at physiological pH values. The solubilty of phytate-mineral complexes is sometimes used as a determinant of mineral bioavailability, however, not all soluble

complexes are bioavailable.

The formation of insoluble phytate salts depends on a number of factors including pH, level and source of phytic acid, and concentration of minerals. Magnesium and calcium salts of phytic acid tend to be soluble at lower pH and insoluble at higher pH (Grynspan and Cheryan, 1983). The solubility of canola phytate has been investigated by Alli and Houde (1987). Canola phytate was found to be insoluble in water, highly soluble under acidic conditions and relatively insoluble under neutral and alkaline conditions. This may indicate that canola phytate is predominantly in the form of metal phytates, particularly calcium and magnesium salts. The solublity profile of wheat and corn appears to be quite different (Figure 2) and may indicate the presence of sodium and potassium phytate or lack of metal-phytate complexes. The differences in phytate solubility among feedstuffs may be an integral component in determining phytate hydrolysis in the gastrointestinal tract of animals.

The formation of metal phytate complexes not only depends on pH but also the molar ratio of mineral to phytic acid. Grynspan and Cheryan (1983) investigated the effects of pH and molar ratio on the formation of calcium phytate complexes. Calcium and phytic acid (Ca:PA) molar ratios ranged from 0.5 to 12.7 and the pH ranged from 2 to 11. They demonstrated that: (1) calcium and phytate phosphorus were highly soluble below pH 4 at all molar ratios, (2) above pH 4 the extent of solubilty drop depends upon the calcium:phytic acid ratio, (3) above pH 6 the greatest calcium precipitation occurred at molar ratios between 4 and 6.5, (4) phytate phosphorus solubility decreased with increasing calcium:phytic acid with complete precipitation occuring above molar ratio of 5 (Figure 3a and 3b), and (5) pentacalcium phytate salt predominates when calcium is not limiting.

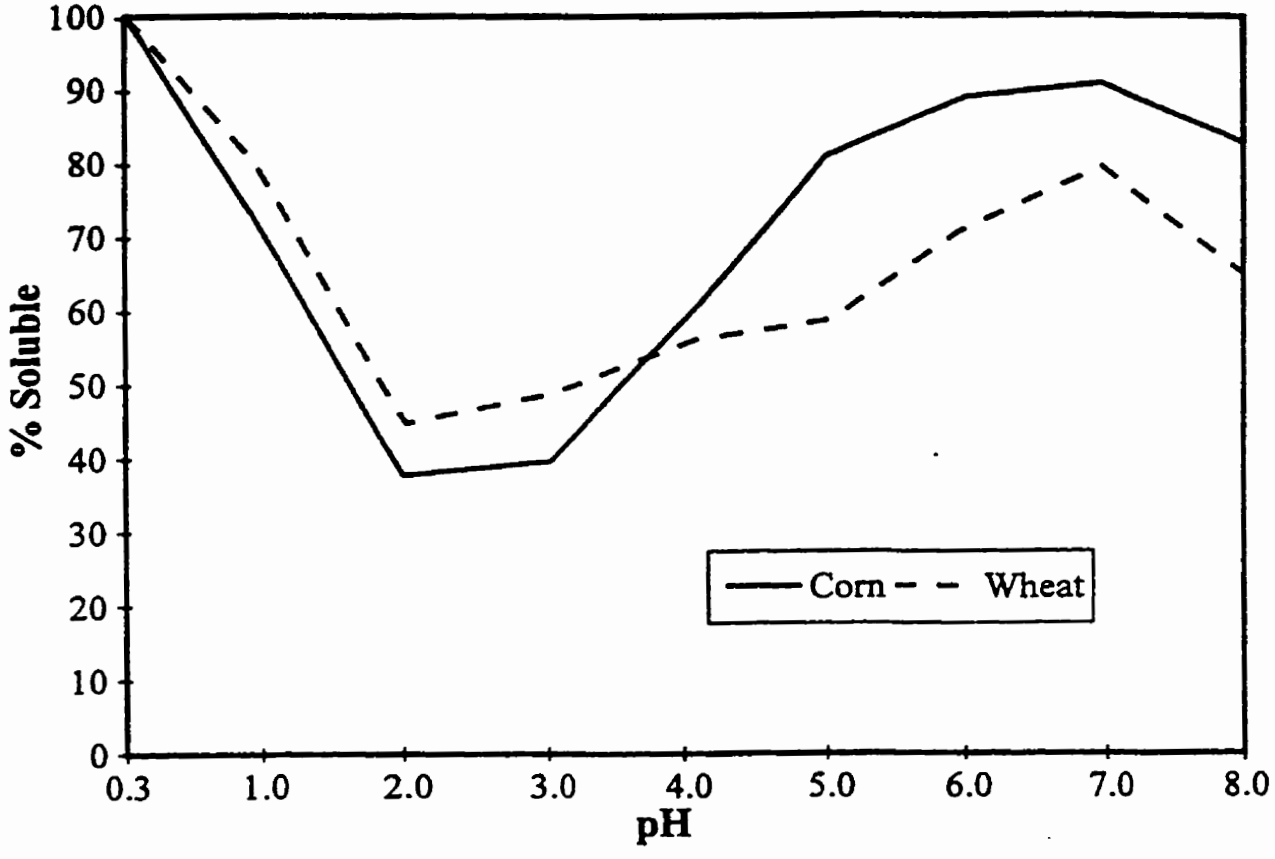


FIGURE 2. Solubility of wheat and corn phytate at various pH values (From Scheuermann et al., 1988)

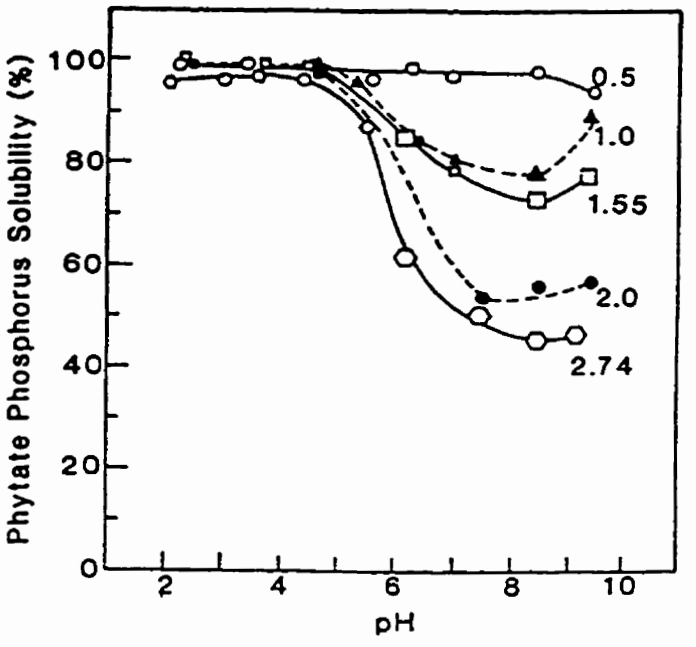


FIGURE 3a. Effect of pH and Ca:PA molar ratio on solubility of phytate phosphorus at low molar ratios of 0.5 to 2.74 (From Grynspan and Cheryan, 1983).

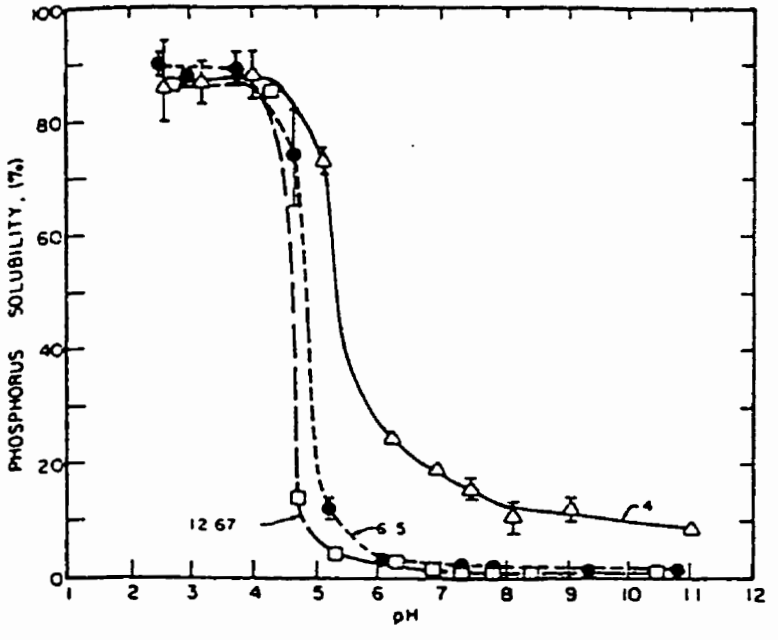


FIGURE 3b. Effect of pH and Ca:PA molar ratio on solubility of phytate phosphorus at high molar ratios of 4.0 to 12.67 (From Grynspan and Cheryan, 1983).

However, Graf (1983) demonstrated that phytic acid exhibits a high affinity for Ca^{2+} over a wide pH range. The study revealed the chelation of Ca^{2+} by phytate at an acidic pH of 2.0 and that certain Ca-phytate complexes were soluble. Molecules of phytate which contain one or two calcium ions are termed mono- and dicalcium complexes, respectively. These complexes are found to be soluble whereas the tri-, tetra-, penta-, and hexacalcium complexes are insoluble. The solubility of these complexes is also shown to be dependent on the calcium:phytate ratio. When large amounts of calcium are present, penta- and hexacalcium complexes are formed. This suggests that there may be substantial binding of cations to phytate in the stomach and the possibility that all metal phytate complexes at a low metal to phytate ratio may be soluble at intestinal pH. The formation of these soluble metal-phytate complexes may facilitate the precipitation of the complex during the subsequent passage through the intestine. Therefore, solubility behavior may not be a good indicator of the presence of metal-phytate complexes since metal ions may bind with phytate yet remain soluble.

The solubility of metal ions and phytic acid is further complicated in the presence of secondary cations. The interaction between zinc and calcium in phytate solutions has been studied. Byrd and Matrone (1965) found that at wide Ca:Zn ratios (high Ca, low Zn) there was increased insolublity of zinc phytate, in other words, calcium enhanced incorporation of zinc into phytate. As the ratio became more narrow (ie. calcium became limiting), the amount of Zn precipitated decreased. Gifford-Steffen and Clydesdale (1993) reported similar results observing increased soluble zinc and phytate at low calcium levels. This suggests that at high calcium levels, the conditions are more suitable for the formation of insoluble Ca-Zn-phytate.

Phytate has been shown to negatively influence mineral bioavailability in a number of species including humans, rats, and poultry. Metabolic balance studies with adult men have indicated that the apparent absorption of calcium decreases when diets high in phytate are consumed (Morris and Ellis, 1985). In poultry, high levels of phytate have also been shown to affect the requirement for calcium. Nelson et al. (1968a) demonstrated that the apparent calcium requirement of White Leghorn chicks increases as the phytate concentration in the diet is elevated. The calcium requirement of chicks fed a diet containing no phytate was 0.5%. The requirement increased to 0.95% by feeding a diet containing 1.25% phytic acid. It has also been shown that growing broiler chicks required more calcium to produce a given level of bone ash when the phytate content of the diet was increased (Farkvam et al., 1989)

The effect of phytate on zinc bioavailability has been extensively researched. High phytate diets affect zinc bioavailability since it forms a highly insoluble salt at pH 6.0 (Maddaiah et al., 1964b), the approximate pH of the intestine where mineral absorption occurs. Feeding rapeseed flour with 2.1% phytic acid to rats caused a reduction in weight gain and feed consumption and decreased plasma zinc levels (Anderson et al., 1976). The addition of 300 ppm zinc to the diet prevented the decrease in feed consumption, weight gain, and plasma zinc levels. O'Dell et al. (1964) reported that the addition of phytic acid markedly decreased the biological availability of zinc in broiler chicks. The study also revealed that excess calcium in the presence of phytate aggravated the zinc deficiency symptoms.

Studies with other minerals have been much less extensive. Magnesium availability was shown to be affected by the addition of phytic acid to a broiler diet containing 75 ppm supplemental magnesium (McWard, 1969). Decreased growth and increased mortality was

observed in broiler chickens fed diets containing 4% phytic acid, however, these problems could be overcome through the addition of 166 ppm magnesium.

Studies on the effect of phytic acid on mineral availability may be difficult to interpret in some cases. Phytic acid can be considered a component of dietary fiber, consequently, plant materials which are high in phytic acid also contain appreciable amounts of fiber. Crude fiber is known to affect mineral bioavailablity (Nwokolo and Bragg, 1977; Torre et al., 1991). It is thus difficult to attribute the adverse effects of phytate on mineral utilization solely to phytate unless it is separated from its fiber components.

Phytic acid-protein interactions

The binding of phytic acid with proteins (either plant storage proteins or digestive enzymes) further complicates the study of phytic acid interactions. Phytic acid is naturally associated with proteins in the aleurone layers of cereal grains and the protein bodies of oilseeds.

The formation of phytate-protein complexes has been shown to be dependent on pH (Cheryan, 1980). At low pH, below the isoelectric point, proteins have a net positive charge due to the basic amino acid residues lysine, arginine, histidine, and the α-amino terminal group. Phytic acid binds proteins as a result of strong electrostatic attraction between the cationic residues on the protein and the anionic phosphate ester of phytate (Figure 4). At intermediate pH, above the isoelectric point, both phytic acid and protein are negatively charged. In this state, phytate complexes with protein in the presence of divalent cations. These divalent cations (usually Ca, Mg, or Zn) simultaneously bind to the protein and phytic

acid molecule and act as a bridge to form a protein-mineral-phytate complex (Figure 5). Evidence of these interactions were demonstrated in a study conducted by Prattley et al. (1982) in which phytate and bovine serum albumin were used to investigate the mechanism of protein-phytate binding. It revealed that under acidic conditions, the protein formed an insoluble complex with phytic acid. In the absence of calcium, the amount of bound phytate decreased with increasing pH; at the same time bound phytate increased in the presence of calcium (Figure 6), implicating that a salt linkage is involved in the interaction. Much less is known about phytic acid-protein interactions at high pH values. There are indications that when the pH is high, the interactions between phytic acid and protein are diminished.

The interactions of phytic acid with proteins may affect the solubility, digestibility, and functionality of proteins. These interactions may be further complicated with the addition of minerals, level and source of phytic acid, and pH which leads to many conflicting results in the literature.

Solubility studies may aid in determining how phytic acid behaves in the gastrointestinal tract. There has been much research centred around phytate-protein solubility as a method to reduce phytate in protein concentrates for human food consumption. Okubo et al. (1976) examined the binding properties of phytic acid to a major soy fraction, glycinin, at various pH values. At pH values of 6, 8, and 10 no binding was observed, however, between pH 5.0 and pH 2.5 binding resulted in insoluble complexes. Serraino and Thompson (1984) studied the effect of pH, heat treatment, and EDTA on the disruption of protein-phytate complexes in rapeseed flour (RF) and rapeseed protein concentrates (RPC). The greater losses of phytate observed with RF as compared to RPC was attributed to the effect

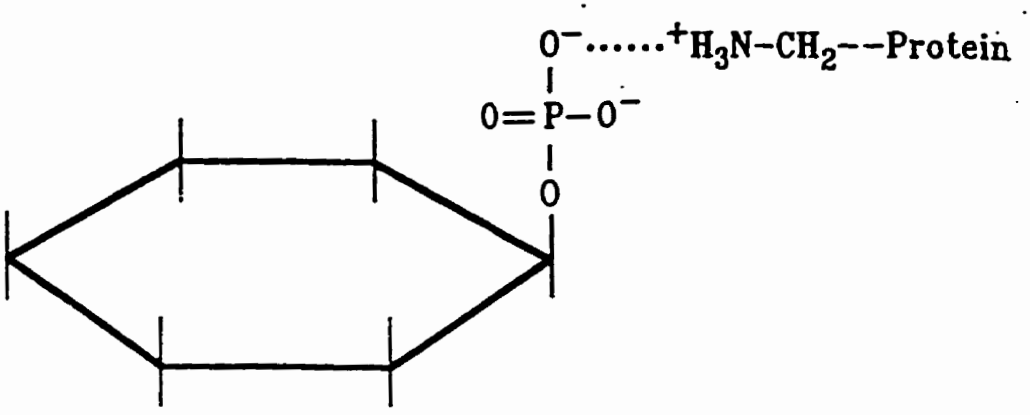
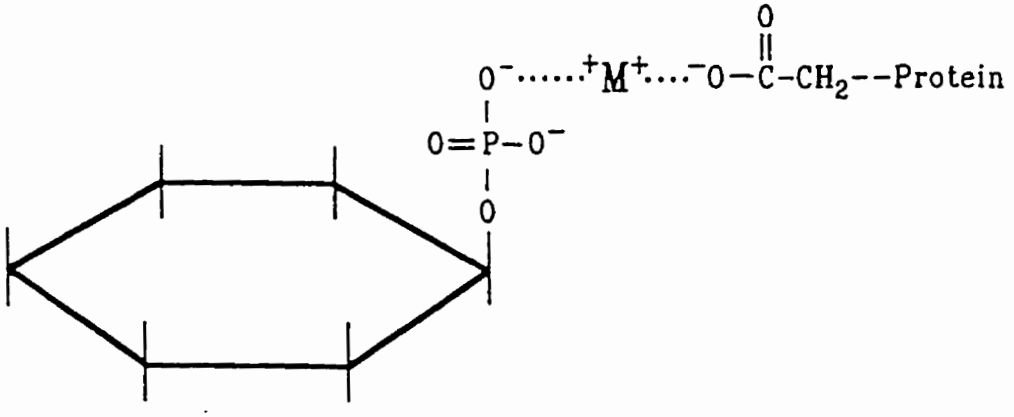


FIGURE 4. Possible structure of phytic acid-protein complex at low pH (From Cheryan, 1980).



M = multivalent cation

FIGURE 5. Possible structure of phytic acid-protein complex at alkaline pH (From Cheryan, 1980).

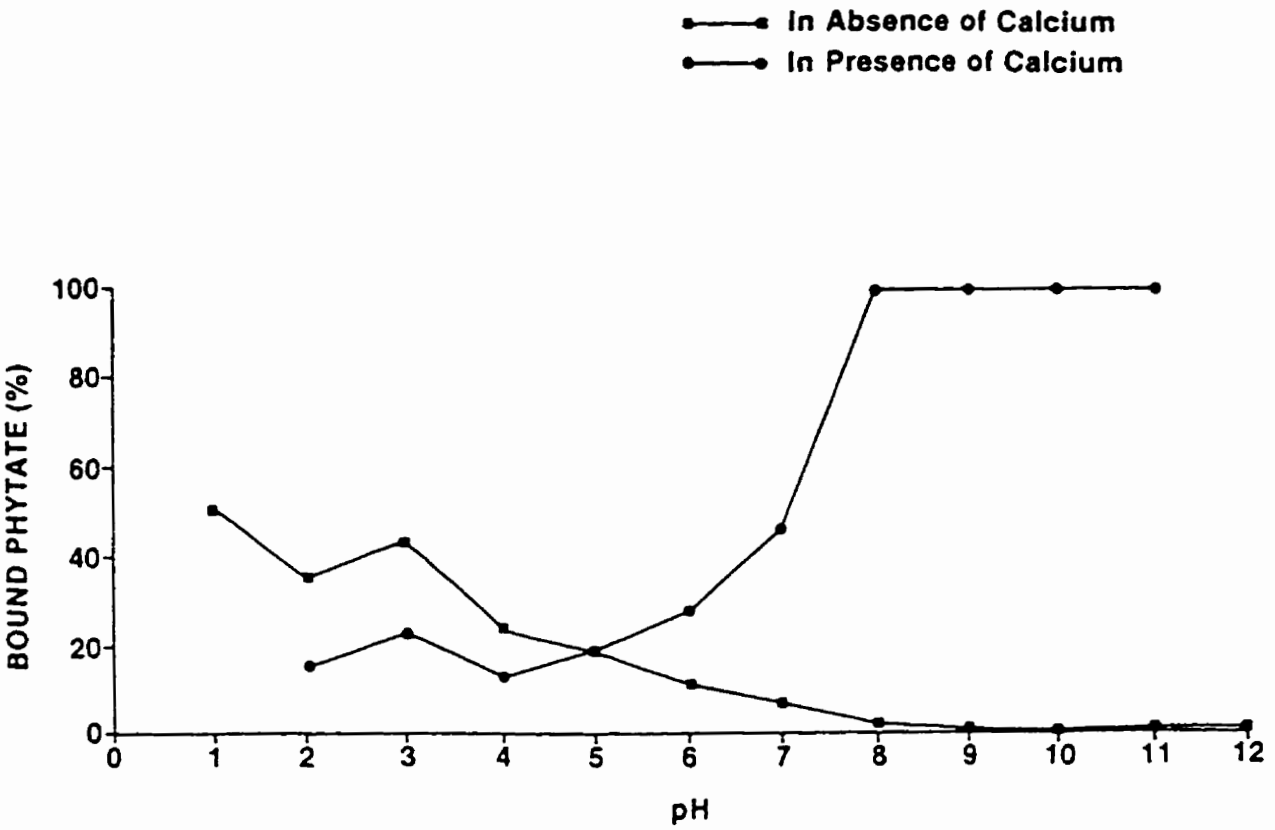


FIGURE 6. Protein bound phytate as a function of pH in the presence of 2% calcium (From Prattley et al., 1982).

of heat treatment. The heat treatment involved in the production of RPC may have uncovered basic amino acid residues which increased the strength of electrostatic interactions with the negatively charged phytic acid rendering a more stable insoluble complex. The addition of EDTA enhanced phytate losses suggesting the occurrence of a ternary protein-mineral-phytate complex at alkaline pH. Cations preferentially bind to EDTA rather than phytate (Cheryan, 1980) forming a soluble cation-EDTA complex, thus, preventing formation of a protein-mineral-phytate complex at alkaline pH.

The binding of phytic acid with proteins may affect the digestibility of proteins and amino acid availability. *In vitro* studies have shown that insoluble protein-phytate complexes are less subject to proteolytic enzymes. Knuckles et al. (1985) studied the effect of sodium phytate on pepsin digestion of casein and bovine serum albumin under *in vitro* conditions. They found that the inhibitory effect of phytate differed with substrate and increased with phytate level. At the highest phytate level, the digestion of casein and bovine serum albumin was reduced by 14% and 7%, respectively. On the contrary, studies with rapeseed flour found that a reduction in phytic acid had no significant effect on *in vitro* protein digestibility (Serraino et al., 1985). Thompson and Serraino (1986) also studied the effect of phytic acid reduction on the *in vivo* digestibility of proteins and absorption of amino acids in rapeseed flour. No significant differences were observed between the rats fed high (5.7%) and low (2.4%) phytic acid diets. This is in agreement with McDonald et al. (1978) who found that the nutritive value of rapeseed protein is not affected by phytic acid level.

Recent studies with phytase indirectly suggest that phytate-protein interactions interfere with protein and amino acid digestibility. Biehl and Baker (1997) found that amino

acid utilization in chicks fed SBM can be improved with exogenous phytase. It is suggested that phytase releases amino acids or gut proteolytic enzymes that are bound to phytate.

Digestive enzymes are known to be negatively influenced by phytate. Singh and Krikorian (1982) found that the *in vitro* activity of the proteolytic enzyme trypsin using casein as the substrate was substantially inhibited by low levels of phytic acid. Phytic acid and calcium was also shown to affect the activation of trypsinogen and the stability of trypsin (Caldwell, 1992). If these conditions are reproduced *in vivo* it may help to explain the decrease in protein digestibilty at high phytate levels.

Phytate decreased *in vitro* starch digestion by α-amylase with the greatest inhibition occurring at pH 4.15 (Knuckles and Betschart, 1987). The decreased α-amylase activity may involve protein precipitation or phytate binding to the active site. At pH 6.0 the inhibitory effect of phytate on starch digestion was not as pronounced or consistent. This may be due to the lack of divalent cations to form protein-cation-phytate complexes, since the protein would have a net negative charge at this pH. Deshpande and Cheryan (1984) found that phytate inhibition of amylase activity was influenced by level of phytate and calcium and magnesium ion concentration. The dependence of amylase activity on preincubation time with phytate as well as phytate concentration is shown in Figure 7. Amylase ativity was lowered by 16% at 0.5 mM phytate and by about 95% at 6.0 mM phytate after 15 min preincubation. The rate of wheat starch digestibility in the presence of phytic acid was determined in an *in vitro* system by Thompson and Yoon (1984). The addition of phytic acid reduced starch digestibility by 60%. It is suggested that phytic acid affects starch digestibility through interaction with amylase enzyme, with protein which is closely associated with starch, directly

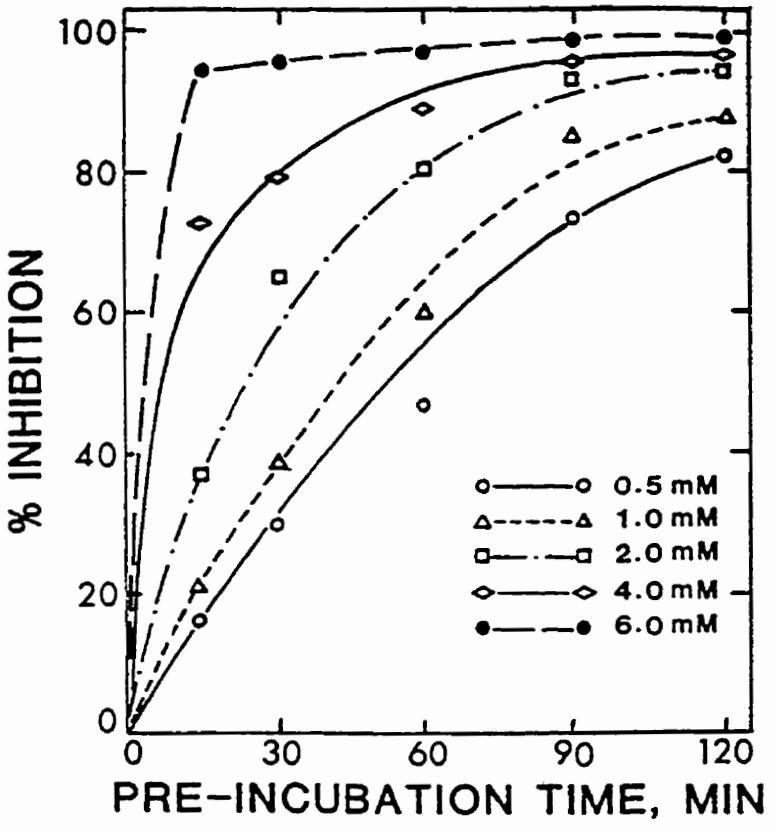


FIGURE 7. Effect of preincubation time and concentration of phytate on amylase activity (From Deshpande and Cheryan, 1984).

with starch, or due to binding with calcium which is known to catalyze amylase activity (Deshpande and Cheryan, 1984).

Availability of phytate phosphorus - General considerations

Over the past 50 years there has been much research conducted on the availability of phytate P to poultry, however, the results and conclusions on the subject remain conflicting. Early studies found that natural phytate was a poor source of phosphorus for poultry (McGinnis, 1944), however, more recent research indicates that adult poultry are able to utilize a good portion of dietary phytate P (Scheidler and Sell, 1987).

Nelson (1976) found that phytate P in corn/SBM and corn/wheat/SBM diets was very poorly utilized. The phytate P recovered in the faeces of the 4 and 9 week old broiler chickens and laying hens fed the chromic oxide diet containing corn was 100, 98, and 92%, respectively. When the wheat was substituted with corn the phytate recovery was 92, 87, and 87%, respectively. This indicates that 0-13% of phytate P was retained. Similarily, Matyka et al. (1990) reported that the retention of phytate P in 3 week old broiler chicks was 5.4% and increased to 17.3% at 7 weeks of age. In contrast, balance experiments conducted by Temperton and Cassidy (1964) showed that the utilization of phytin P by the chick fed a diet containing wheat, corn, and SBM was about 60%. Likewise, Edwards (1982) found that phytate P retention was quite high (35-60%) in corn/SBM diets for Leghorn and broiler chickens. In a series of experiments conducted by Waldroup et al. (1964) the P bioavailability of inorganic phosphorus supplements was compared to sodium phytate, calcium phytate, and chemically isolated phytic acid. Phosphorus from phytic acid was found

to be as available to the chick as dicalcium and monocalcium phosphate. Sodium phytate P was slightly less available than phytic acid P whereas calcium phytate was highly unavailable.

The utilization and availability of P varies widely among feedstuffs. The requirements for P are expressed in terms of non-phytate P (nP), that is, P which is not associated with phytate and is thought to be highly available. For most feedstuffs, 30 to 40% of total P is considered to be available (NRC, 1994). Based on the above recommendations this may not be an accurate method to express requirements since poultry are known to utilize a portion of phytate P. Nwokolo and Bragg (1980) found the availability of P in rapeseed meal to be 75.3%. These results are much higher than the availability of 30 to 40% usually associated with P from plant origins. Rapeseed meal (or canola meal) is high in P content (1.17%, NRC 1994) and when included in poultry diets could make a significant contribution to the P requirement. The availability of organic P in corn, wheat, and barley was estimated by Hayes et al. (1979) using tibia breaking strength as a criteria. The availability of P in corn, hard wheat, soft wheat, and barley was reported at 12, 43, 58, and 50%, respectively. The greater availability of P in wheat and barley *vs.* corn was attributed to the higher amount of natural endogenous phytase in these grains.

In general, there is a lack of consistency in experimental data on the extent of phytate P utilization by poultry. The factors influencing phytate hydrolysis are outlined by Ravindran et al. (1995a) and may help to explain the wide discrepancy among researchers. These factors include: source of phytate, age of birds, levels of calcium and vitamin D_3, method of phytate analysis, experimental techniques, and criteria of response measured. Some dietary factors influencing phytate hydolysis will be described in further detail in the following section.

The effect of calcium on phytate P availability

Calcium is known to influence the hydrolysis and absorption of phytate P in poultry. The availability of phytate P is reduced at high calcium levels due to the formation of an insoluble calcium-phytate complex (Wise, 1983) which prevents enzymatic hydrolysis.

Ballam et al. (1985) studied the chicks ability to hydrolyze phytate at different dietary levels of calcium and non-phytate P. Three week old broiler chicks were fed corn-soy diets containing varying amounts of calcium (.09 or 1.0%) and non-phytate P (.12 or .45%). They found that increasing the calcium content decreased phytate hydrolysis regardless of non-phytate P content (Table 2). However, the calcium level of .09% is too low for optimum performance of birds and has no practical significance. Early studies by Nott et al. (1967) demonstrated that the extent of phytate P utilization was largely determined by level of calcium in the diet. The investigators found in laying hens fed calcium levels of 1.5, 2.5, 3.5, and 4.5% that phytate P retention was 39, 16, 6, and 5%, respectively. Thus, under practical feeding conditions phytate P is very poorly utilized. Ballam et al. (1984) reported that chicks consuming diets containing .83% calcium hydrolyzed more phytate than those fed 1% calcium. Scheidler and Sell (1987) reported that phytate P retention in laying hens decreased as calcium levels increased from 3.0 to 4.0%.

The relationship of Ca:P ratios to utilization of phytate P has been examined. Vandepopuliere et al. (1961) found that chicks fed diets containing 0.28% plant phosphorus with Ca:P ratios of 4:1 and 8:1 developed severe rickets, however, when the ratio was narrowed to 2:1 and 1:1 the severity of rickets decreased. Chicks fed a diet with a Ca:P ratio of 1:1 performed better in growth than chicks fed a 2:1 Ca:P ratio. Studies by Harms et al.

TABLE 2. Effect of dietary levels of calcium and non-phytate phosphorus on phytate[1] hydrolysis by chicks

Non-phytate phosphorus (%)	Calcium (%)	Phytate hydrolysis (%)
0.12	0.09	42.1
0.12	1.00	8.3
0.45	0.09	56.8
0.45	1.00	5.9

[1]Dietary phytate P level was constant at approximately .23%

(From Ballam et al., 1985)

(1962) revealed that phytic acid is available for growth and bone calcification provided the Ca:P ratio is in balance. Widening the Ca:P ratio from 1:1 to 2:1 decreased the availability of phosphorus in phytic acid to a greater extent than that from dicalcium phosphate.

The effect of vitamin D on phosphorus availability

The general function of vitamin D is to elevate plasma Ca and P to a level that will support normal mineralization of bone. This is accomplished by stimulating specific pump mechanisms in the intestine, bone, and kidney (DeLuca, 1979).

Early investigators reported that phytate P utilization is low in rats fed diets deficient in vitamin D (Pileggi et al, 1955). More recently, Mohammed et al. (1991) demonstrated that raising cholecalciferol levels dramatically increased phytate digestibility and the retention of Ca and P in broiler chicks. Phytate P digestibility increased from 50 to 76.5% through increased cholecalciferol intake. The use of 1,25-dihydroxycholecalciferol (1,25-OH_2 D_3), the active metabolite of vitamin D_3, in poultry diets has been actively researched. Edwards (1989) reported that the addition of 1,25-OH_2 D_3 to broiler chicken diets caused a significant increase in bone ash and a decrease in tibial dyschondroplasia. In another study Edwards (1993) determined that the supplementation of a corn/SBM diet with 1,25-OH_2 D_3 and phytase resulted in increased weight gain and bone ash, lowered incidence of rickets, and improved retention of phytate P.

Vitamin D_3 may increase the availability of phytate P by one or more of the following mechanisms as outlined by Ravindran et al. (1995a). First, it is thought that vitamin D_3 increases synthesis or activity of intestinal phytase. Davies et al. (1970) found that the activity

of phytase increased when a phosphorus deficient diet was supplemented with 750 and 7500 ICU of vitamin D_3/kg of diet. Secondly, vitamin D may increase phytate hydrolysis via stimulation of calcium absorption, rendering the phytate more soluble and available for utilization (Wise, 1983). Finally, it is well documented that vitamin D_3 enhances transport of P. Studies by Wasserman and Taylor (1973) have demonstrated that vitamin D_3 stimulated ^{32}P translocation in all segments of the chick small intestine. It is likely that no one mechanism is more prevalent than the other, rather it is the functioning of all three mechanisms which leads to enhanced utilization of phytate P.

Phytase enzyme and phytate hydrolysis

For phytate to be utilized by poultry, the orthophosphate molecules must be cleaved from the inositol ring of phytic acid and subsequently absorbed. This dephosphorylation is catalyzed by the enzyme phytase (myo-inositol hexaphosphate phosphohydrolase), which is a specific phosphatase which hydrolyzes the stepwise removal of a phosphate ester from a phytic acid molecule. This hydrolysis can occur within the GI tract or in the feed prior to consumption.

There are two main types of phytase: a 3-phytase (EC 2.1.3.8) in which dephosphorylation of phytate is initiated at the 3 position of myo-inositol or a 6-phytase (EC 3.1.3.26) which commences at position 6 (IUPAC-IUB, 1976). 3-phytase is generally produced by microorganisms whereas 6-phytase is found in plants. Consequently, the mode of action of these enzymes is quite different. Wheat phytase activity was compared to the microbial phytase enzyme preparation, Natuphos®, in a study by Eeckhout and De Paepe

(1996). The researchers found that wheat phytase and microbial phytase (Gb phytase) had similar activities at pH 5.5 *in vitro* (Figure 8). When pH was lowered to 3.0, the microbial phytase was very active (80% of its activity at optimum pH 5.5) while wheat phytase was no longer active. This may have ramifications in determining regions of the GI tract in which endogenous and microbial phytase will be active *in vivo*.

The dephosphorylation of a phytate molecule occurs in a well defined manner. Kies (1996) illustrates that phytase does not degrade a phytate molecule (inositol-6-phosphate) as far as possible followed by the next phytate, rather that first one phosphate group is cleaved off from many molecules, resulting in inositol-5-phospha.e. Then the next phosphate group is cleaved off resulting in inositol-4-phosphate molecules, and so on.

Sources of phytase enzyme

There are three possible sources of phytase in the gastrointestinal tract: the intestine; the bacterial microflora of the gut; and the diet, which may contain endogenous plant phytase or exogenous microbial phytase. These three sources will be examined in the following section.

Intestinal phytase

Much controversy exists regarding the presence of intestinal phytase in poultry. Phytase activity was demonstrated in the extracts of the mucosa of the small intestine of the chicken by Bitar and Reinhold (1972). Likewise, Davies and Motzok (1972) isolated a phytase in chick intestinal mucosa which would hydrolyze sodium phytate. Data from other

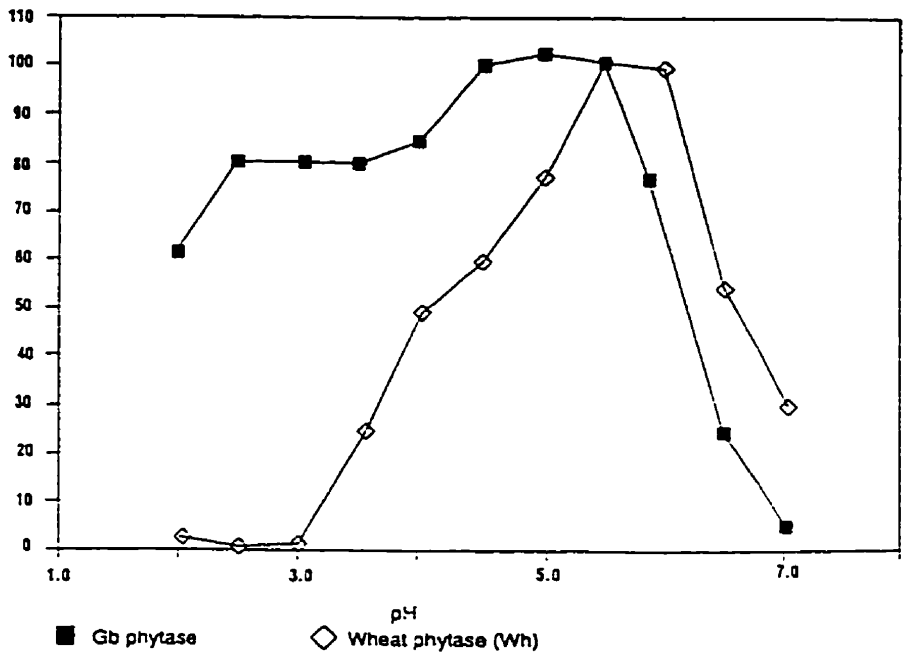


FIGURE 8. Influence of pH on phytase activity (From Eeckhout and De Paepe, 1996).

studies remain conflicting as to whether phytase is an independent enzyme or whether phytate hydrolysis is attributable to other phosphatases. Maddaiah et al. (1964a) concluded that the phytase activity obtained from homogenates of chick intestinal mucosa was due to a non-specific phosphatase. Researchers from the University of Saskatchewan found that the small intestinal brush border membrane of broiler chickens and laying hens contains a phytase activity that is distinct from non-specific acid and alkaline phosphatases (Maenz et al., 1997). The specific activity of phytase was highest in the duodenum and lowest in the ileum with a linear decrease in activity down the length of the gut. Intestinal phytase and alkaline phosphatase were shown to be regulated by the same factors in studies by Moore and Veum (1983) and McCuaig et al. (1972) indicating that both enzymes are involved in phytate hydrolysis. Thus, much of the data on intestinal phytase remains unclear, however, it appears that there is minor endogenous phytase activity in the intestinal mucosa of poultry which contributes to phytate hydrolysis.

Microbial phytase

Phytase is produced by fungi, bacteria, yeast, and soil microorganisms (Reddy et al., 1989). It is also well established that the microflora of the rumen produces phytase which effectively hydrolyzes phytate in plant materials. Consequently, ruminants are able to utilize the majority of phytate present in their diets. Research conducted on high producing dairy cows found that they could hydrolze 98% of dietary phytate (Clark et al., 1986).

It is unknown if bacterial phytase in poultry contributes to the hydrolysis of phytate. Under *in vivo* conditions it is difficult to determine the contribution of intestinal or microbial phytase. Warden and Schaible (1962) investigated the addition of lysed *E.coli* to corn/SBM

diets deficient in P. The results indicated that the addition of lysed *E.coli* produced normal growth and substantially improved bone development. This suggests that enzymes, possibly phytase, are released from *E.coli* and are active in the digestive tract of poultry. However, it is questionable if microbial phytase is active in areas of the small intestine where P absorption occurs (proximal small intestine). It must be remembered that phytate hydrolysis is not indicative of P absorption and utilization.

Dietary phytase

Poultry diets may contain phytase from two sources: either endogenous plant phytase or supplemental exogenous phytase. It has long been known that various plant feedstuffs contain high amounts of endogenous phytase activity. The phytase activity of various feed ingredients are outlined in Table 3. Wheat, barley, and rye are considered to be high in phytase activity whereas corn and oats contain little or none. Oilseeed meals contain low levels of phytase activity due to heat treatment during processing. The phytase activity in wheat and rye by-products is extremely high since the majority of phytase is located in the bran portion of these feedstuffs. It has been suggested that the addition of dietary ingredients high in phytase activity to pig and poultry diets will improve phytate P availabilty (Jongbloed and Kemme, 1990; Pointillart et al., 1984).

Many of the factors influencing phytate content in feedstuffs also influence the level of phytase. These include variety, location, fertilization, year, and storage conditions (Nys et al., 1996). Variety appeared as a significant factor explaining the differing endogenous phytase activity of various wheat varieties in France (Barrier-Guillot et al., 1996). The altered phytase activity observed in vitro between different sizes of wheat particles also suggests that

the extent of grinding may affect phytase activity (Figure 9).

A loss of phytase activity may occur during the pelleting of feeds. Pelleting temperatures vary from 70 to 80°C or higher in some cases. At these temperatures a certain amount of plant phytase will be denatured which results in a loss of phytase activity. Pointillart (1993) found that the processing of feeds containing high phytase activity (wheat and rye), including steam treatment, does not alter this activity at temperatures between 47 and 62°C, however, heating to 70-80° C causes partial or total inactivation. Concurrently, Jongbloed and Kemme (1990) have shown that steam pelleting (80°C) of diets containing considerable phytase activity resulted in reduced P availability in pigs due to the loss in phytase activity.

It is questionable as to whether endogenous plant phytase can withstand low pH conditions. As mentioned previously, plant phytases have pH optima of 5 to 5.5 and are virtually inactive at pH values below 3 (Eeckhout and De Paepe, 1996). Hill and Tyler (1954) found that the endogenous plant phytase enzyme is irreversibly destroyed when submitted to pH values below 2.5, hence, in the GI tract of pigs and poultry, endogenous plant phytase would become inactive in the stomach. If this is the case, the addition of feedstuffs high in phytase activity would have little or no effect on improving P availability. However, various studies have illustrated that the addition of such ingredients to pig and poultry diets improves phytate P digestibility and P availability. A study with pigs conducted by Pointillart et al. (1984) examined the effect of two cereal-based diets (wheat and corn) on the utilization of P and intestinal phytase activity. Phosphorus utilization of wheat fed pigs was 1.7 times that of corn-fed pigs. This was attributed to higher dietary phytase activity of wheat, since the

TABLE 3. Phytase activity of various feed ingredients

Feedstuff	Phytase activity (U/kg)
Rye	5130
Wheat bran	4601
Wheat	1193
Barley	582
Oats	42
Corn	15
Soybean meal	40
Canola meal	16

(From Eeckhout and De Paepe, 1994)

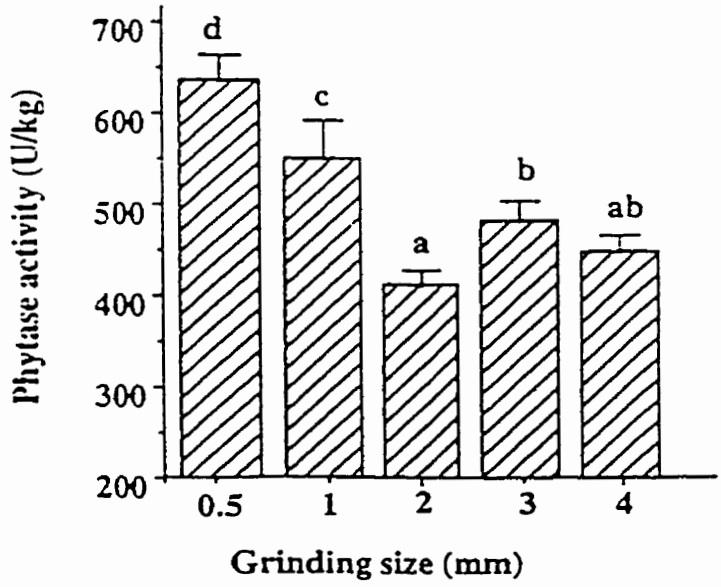


FIGURE 9. Effect of grinding size on the measured phytase activity of wheat (From Nys et al., 1996).

intestinal phytase activity and P content of the diet were the same for all pigs. The addition of triticale, a hybrid of wheat and rye, to laying hen diets was found to increase the availablility of P in corn and SBM making inorganic phosphate supplementation almost unnecessary (Sauveur, 1984).

In the past 10 years, the use of exogenous phytase preparations in feed has become common in areas of intensive animal production in an attempt to reduce P excretion. These phytase preparations are obtained by fermentation of genetically modified microorganisms, usually the *Aspergillus niger* strain. The phytase preparation may be added to diets in liquid or powder form. The best example of such a preparation is Natuphos® developed by Gist-Brocades (Delft, The Netherlands) and marketed by BASF Corp (Mount Olive, NJ, USA 07828-1234). The activity of phytase is measured in units (U) or FTU (fytase units). One unit of phytase activity is defined as the amount which liberates 1 micromol of inorganic phosphorus per minute from an excess of sodium phytate at 37°C and pH 5.5. The advantage of using microbial phytase is that known amounts of enzyme activity can be added to diets. In addition, exogenous phytases are active over a wide pH range. In this regard, Natuphos® phytase has two pH optima at pH 2.5 and pH 5.5 (Heinzl, 1996). Due to its low pH optima of 2.5, Natuphos® phytase is considered to be active in the stomach of pigs and proventriculus of poultry. Kornegay and Yi (1996) found that when phytase (750 and 1500 U/kg) was added to diets of pigs most of the phytase activity was in the stomach (30-85% of diet phytase activity) and upper small intestine. A similar pattern was observed in chickens fed diets containing 500 and 1000 U/kg of phytase. The highest level of activity was observed in the crop (69-86% of diet phytase activity) with about a 50% reduction in the

proventriculus. No activity was detected in the small intestine.

Use of exogenous supplemental phytase in poultry diets

In recent years, due to increasing environmental concerns, there has been considerable interest in the use of supplemental phytase in poultry diets. Supplementing feeds with phytase has been an effective tool in improving P availability and thereby reducing the amount of P in animal manure. Also, less inorganic P (which is expensive) needs to be added to the diet and studies suggest that mineral and amino acid availability are improved (Biehl and Baker,1997; Yi et al., 1996b).

About 30 years ago, Nelson et al. (1968b) were the first to show that a phytase produced by *Aspergillus ficuum* and other molds hydrolyzed the phytate in SBM In this study SBM was pre-treated with a phytase preparation and then fed to chicks. They found that chicks utilized phytate P as efficiently as they did inorganic P, whereas the chicks fed the untreated SBM did not utilize the phytate P as effectively. In a follow-up study, Nelson et al. (1971) added a fungal phytase directly to a corn/SBM diet deficient in P. The addition of phytase to the diet produced an increase in percentage bone ash indicating hydrolysis of phytate in the alimentary tract of the chicken. Likewise, the treatment of soybean and cottonseed meals with *Aspergillus ficuum* has shown that 63-85% and 42-67% of the phytate was hydrolyzed, respectively (Han and Wilfred, 1988; Han, 1989). Zyla et al. (1995) used various preparations of phytase to study the extent of corn/soybean feed dephosphorylation *in vitro* under simulated intestinal conditions of the turkey. The phytase enzyme preparations were shown to completely dephosphorylate phytate present in corn/soybean feed.

More recently, the supplementation of microbial phytase to corn/SBM diets has resulted in marked improvements in P availability in broilers (Kornegay et al., 1996), turkeys (Ravindran et al., 1995b), and laying hens (Simons and Versteegh, 1993). Simons et al. (1990) found that when microbial phytase was added to low P diets for broilers the availability of P increased to over 60% with growth rate and feed conversion comparable to or even better than those obtained on control diets. In a study conducted by Broz et al. (1994), phytase supplementation increased mean weight gains and feed intake of chickens by 6.5-13.0% with a moderate improvement in feed to gain ratio. The effectiveness of supplemental phytase in improving the availability of phytate P in SBM was examined by Denbow et al. (1995). Seven levels of phytase (0, 200, 400, 600, 800, 1000, and 1200 U/kg diet) were added to diets containing 0.20, 0.27 or 0.34% non-phytate P (nP). Body weight gains and feed intake were improved by phytase supplementation at all nP levels with the magnitude of response greatest at low nP levels. Gain to feed ratio was unaffected by phytase addition. A high percentage of mortality was observed for chicks fed 0.20 and 0.27% nP diets without added phytase, however, this declined with the addition of 200 to 400 U phytase/kg diet. It was estimated that 31 to 58% of the P from phytate was released depending on level of phytase supplementation. Results demonstrated that 821 U of phytase is equivalent to 1 g of P. Laying hens have also been shown to respond to phytase supplementation. An experiment was conducted on the effects of phytase on the performance of Single Comb White Leghorn laying hens fed corn/SBM diets varying in available P (Boling et al., 1997). The P requirement of the hens was found to be .15% available P based on performance. From 28-36 weeks, the 0.1% available P diet with no supplemental phytase

resulted in significantly lower egg production and egg yield, feed consumption, feed efficiency, body weight and increased mortality. The addition of 300 U of phytase/kg to the 0.1% diet resulted in maximum performance indicating that phytase improved utilization of phytate P in corn/SBM diets.

Histological, mechanical, and chemical properties of toes and tibias from broilers and turkeys may be used as indicators of P availability. Numerous studies have shown that toe ash and tibia breaking strength are increased by dietary phytase (Perney et al., 1993; Yi et al., 1996c; Zyla et al., 1996). Qian et al. (1996b) investigated the effect of supplemental phytase on histological bone development of broilers fed semi-purified diets containing SBM as the only source of organic P. Generally, birds fed low P diets had poor bone development which was characterized by a widened hypertrophy zone, narrower cartilaginous and proliferative zone, and thinner and weaker trabecular bone. Phytase supplementation resulted in increased tibia length, shear force, shear stress, and ash content. This indicates that enzyme supplementation improved bone health by promoting normal bone development and mineralization either through enhanced P availability or release of other trace minerals.

Excess P in animal manure is potentially harmful to the environment. The runoff from fields containing high levels of P enters surface water encouraging growth of algae and other aquatic plants which results in a deterioration in water quality. Regulations are now being imposed in European countries which limits the amount of N and P that can be applied to the land. With rapid expansion of intensive livestock operations, these restrictions may hit parts of the USA and Canada in the near future. This would result in either producers decreasing the number of animals in their units or finding methods to reduce the level of N and P in

manure. The addition of microbial phytase to swine and poultry diets has proven to be an effective means of reducing P excretion. Simons et al. (1990) found that the amount of P in the excreta of pigs and broilers decreased by 35 and 50%, respectively, with the addition of 1500 U microbial phytase/kg diet. Likewise, Yi et al. (1996c) reported that P excretion was reduced 45% by addition of 1050 U microbial phytase to a P deficient (0.27% available P) diet compared to a positive control diet containing 0.45% available P.

An example of the potential benefit of using low P diets supplemented with phytase is shown in Table 4. This table gives an example of a 2 kg broiler with a feed conversion of 2.0, consuming .65 or .50% total P in a 42 day growth period. The amount of P retained is 4.9 g per kg liveweight. If this broiler is fed a diet with .65% total P (.45% available P + .20% phytate P), 26 g of P would be consumed in the 42 day growth period, 9.8 g would be retained and 16.2 g would be excreted. Reducing dietary P to .50% (.30% available P + .20% phytate P) would reduce intake to 20 g of P per growth period and would reduce the amount of P excreted to 10.2 g, a 37% reduction in P excretion. Considering a province such as Manitoba which raises 20 million broilers a year, a 37% reduction in P would result in 120 tonnes less P entering the environment annually. At the present time, Manitoba has a large land base capable of handling much of the manure from livestock operations, however, in provinces such as B.C. and Ontario a reduction of this magnitude could have a major impact on the environment.

Phytate has the ability to bind minerals such as Zn, Cu, Mn, Fe, Mg, and Ca (Maddaiah et al.,1964b). The hydrolysis of phytate by phytase should release these bound minerals making them available for intestinal absorption. Supplementing corn-SBM diets with

TABLE 4. Potential of phytase for reducing phosphorus excretion in a 2 kg broiler.

Phosphorus	Requirement (NRC, 1994)	+ Phytase
Dietary (%)[a]	.65	.50
Intake (g)[b]	26	20
Retained (g)[c]	9.8	9.8
Excreted (g)	16.2	10.2
Reduction in excretion (%)		37

[a]Assumes .20% phytate P
[b]2.0 feed conversion x 2 kg = 4 kg of feed consumed in a 42 day growth period
[c]Assumes 4.9 g of P retained per kg liveweight

microbial phytase improved bioavailability of zinc to weanling pigs (Lei et al., 1993) and broilers (Yi et al., 1996b). A corn/SBM basal diet containing 20 ppm Zn was fed alone and supplemented with 5, 10, or 20 ppm Zn or with 150, 300, 450, or 600 U of phytase/kg of diet in a broiler study conducted by Yi et al. (1996b). Body weight gain, feed intake, DM retention, and Zn retention were increased by adding phytase indicating that phytase is effective in improving Zn utilization in broilers. Aproximately 0.9 mg of Zn was released per 100 U of phytase used. Roberson and Edwards (1994) found that phytase did not affect zinc retention, however, phytase plus 1,25-OH_2 D_3 increased Zn retention synergistically in Zn deficient corn/SBM diets. A broiler experiment designed to study the efficacy of microbial phytase on relative retention of P, Ca, Cu, and Zn and mineral contents of plasma and bone was conducted by Sebastian et a'. (1996a). Phytase supplementation of a low P diet increased the relative retention of total P, Ca, Cu, and Zn by 12.5, 12.2, 19.3, and 62.3 percentage units, respectively. Minor effects were observed on the concentration of minerals in tibia ash and plasma. Microbial phytase was shown to reduce Cu utilization by 50% in chickens fed SBM in a study conducted by Aoyagi and Baker (1995). It was thought that phytase may have increased Zn utilization and antagonized Cu absorption. Biehl et al. (1995) reported that manganese utilization is increased by 1200 U phytase and 10 ug/kg 1,25-OH_2 D_3. The concentration of Mn in bone nearly doubled with supplementation of phytase and 1,25-OH_2 D_3. This improved Mn utilization may aid in combatting leg weakness in poultry.

The addition of phytase to diets may also aid in hydrolyzing protein-phytate complexes liberating protein and amino acids, however, data in this area is scarce. Yi et al. (1996a) investigated the effect of microbial phytase on N and AA digestibility and N retention

in turkey poults fed corn/SBM diets. The treatments were arranged in a 2x2x2 factorial arrangement consisting of 0.45 and 0.60% nP, 22.5 and 28.0% CP, and 0 or 750 U of microbial phytase. The addition of phytase to either 22.5 or 28.0% CP diets increased body weight gain and percentage toe ash. The magnitude of the effect of phytase was less at 0.60% nP than for 0.45%. Apparent and true ileal digestibility of N and AA was increased with phytase supplementation to 22.5% CP diets at both 0.45 and 0.60% nP. Minor improvements (2%) in true amino acid digestibility were reported by Biehl and Baker (1997) when 1200 U/kg phytase was supplemented to SBM and administered to cecectomized roosters. In another study by Biehl and Baker (1996), young pigs fed amino acid deficient corn/SBM diets (15.5% CP) supplemented with 1200 U/kg of phytase showed increased weight gain and feed efficiency, growing as fast and as efficiently as those fed the 19.5% CP positive control diet.

In summary, the supplementation of phytase in poultry diets has been shown to increase phytate P availability and to reduce the negative effects of P excretion into the environment. Studies have also shown that the addition of microbial phytase to diets high in phytate P will enhance mineral and amino acid availability. Although much of the research to date on microbial phytase has been conducted with corn/SBM diets, very little is known on the mode of action of this enzyme when applied to wheat/canola meal-based diets. In Western Canada, canola meal is an important protein supplement and along with being the richest source of available P it is known to contain the highest level of phytate P. Therefore, the addition of phytase to diets containing canola meal may prove beneficial in improving the nutritive value of this feedstuff.

CHAPTER 3

EFFECT OF SUPPLEMENTAL PHYTASE ON PHYTATE DIGESTIBILITY OF WHEAT/CANOLA MEAL DIETS: *IN VITRO* AND *IN VIVO* STUDIES

ABSTRACT

In vitro and *in vivo* studies were conducted to determine the digestibility of phytate in canola meal, wheat, and wheat/canola meal-based diets supplemented with a commercial preparation of phytase enzyme (Natuphos® 5000). An *in vitro* procedure was used to determine the rate of phytate hydrolysis under simulated conditions of the gastrointestinal tract. Complete hydrolysis of phytate in canola meal by phytase was accomplished under the conditions studied. Phytate hydrolysis in wheat was dependent on the degree of grinding with 37 and 61% of phytate hydrolyzed, respectively, in wheat ground to pass through a 5 mm screen (course) and that ground to pass through a 1 mm sieve (fine). Only partial hydrolysis (46%) of phytate was attained *in vitro* when a wheat/canola meal diet containing phytase enzyme was used. In the *in vivo* study, broiler chickens were fed wheat/canola meal-based diets containing two levels of non-phytate P (nP), 0.25 and 0.35%, each supplemented with phytase enzyme to provide 0, 500, and 1000 units/kg diet. A diet containing 0.45% nP served as a positive control. In comparison to the positive control, no differences in feed intake, weight gain, and feed to gain ratio were observed for the 0.35% nP diet regardless of phytase supplementation. The addition of 500 and 1000 units phytase/kg to 0.25% nP diets linearly improved ($P<0.05$) feed intake and weight gain. The high level of phytase supplementation produced responses equal to that of the positive control. Moderate improvements in feed to

gain ratio were noted among dietary treatments. Ileal digestibility of phytate in control diets with no enzyme added, regardless of nP content, averaged 52%. Addition of 1000 units phytase/kg to low nP diets resulted in increased phytate digestibility to 62% ($P<0.05$). Diets containing 0.35 and 0.25% nP reduced total P excretion (g/kg DM) by 24 and 29% ($P<0.05$), respectively as compared to positive control. The addition of phytase to the 0.25% nP diet further reduced total P excretion ($P<0.05$) by 9 percentage points. The results indicate that supplementation of a wheat/canola meal-based diet with phytase enzyme enhances P availability and results in reduced P excretion.

Key words: canola meal, phytate, phosphorus, phytase, broiler chicken

INTRODUCTION

Wheat and canola meal are common feed ingredients in poultry diets in Western Canada. Canola meal is a valuable source of protein (36%, NRC 1994) and amino acids, especially the sulphur containing amino acids . It is also characterized by its high content of phosphorus (P)(1.1%, NRC 1994). Although canola meal is an excellent source of available P it also contains a high level of phytate P (0.78%), a level much higher than that present in many protein supplements including soybean meal (SBM). Therefore, the use of exogenous phytase may aid in improving the nutritive value of this feedstuff. Some studies have already indicated that the use of wheat in pig and poultry diets improves P availability as a result of endogenous plant phytases present in wheat (Pointillart et al., 1984; Usayran and Balnave, 1995; Pointillart, 1993). Therefore, the use of both wheat and microbial phytase in poultry diets may be an effective means of improving phytate P utilization.

It has long been established that the addition of microbial phytase to corn/SBM diets is effective in improving phytate P availability (Nelson et al., 1971). Improvements in body weight gain, feed intake, feed efficiency, and toe and tibia ash along with a reduction in P excretion have been documented in broiler chickens fed corn/SBM diets supplemented with phytase enzyme (Simons et al., 1990; Perney et al., 1993; Broz et al., 1994; Yi et al., 1996c; Kornegay et al., 1996). To our knowledge there is little information on the supplementation of microbial phytase to wheat/canola meal-based diets which are known to contain an appreciable amount of phytate P.

The present experiment was conducted to examine the factors influencing phytate

hydrolysis of wheat/canola meal-based diets supplemented with phytase enzyme. *In vitro* trials simulating the gastrointestinal tract conditions of the chicken served, along with *in vivo* experimentation, as methods to determine the effect of phytase supplementation on phytate hydrolysis, P availability, P excretion, and overall performance of broiler chickens.

MATERIALS AND METHODS

IN VITRO STUDY

An *in vitro* procedure (Slominski, unpublished) developed in our laboratory was used to study the degree of phytate hydrolysis under simulated conditions of the gastrointestinal tract. Phytate hydrolysis in canola meal, wheat ground to pass through a 5 mm screen (course; WC) , wheat ground[1] to pass through a 1 mm sieve (fine; WF), WC /canola meal mixture (2:1 w/w), WF/canola meal mixture (2:1 w/w), and a wheat-canola meal diet (Basal Diet 1, Table 5) were examined. A constant phytate to phytase ratio was employed which provided 1850, 900, 900, 1100, 1100, and 1000 U of phytase (Natuphos® 5000, BASF), per kilogram of sample, respectively. An outline of the solutions and time frames used in the procedure is depicted in Figure 10. The samples were incubated in an incubator-shaker[2] at 40°C and shaken at 200 rpm. After the incubation period, the samples were placed in liquid nitrogen for 2 minutes to stop enzyme activity. Following freeze drying[3] the samples were

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[1]Cyclotec 1093 Sample mill, Hoganas, Sweden
[2]New Brunswick Scientific, Edison, NJ
[3]Virtis, 815 Route 208, Gardiner, NY 12525-9989

FIGURE 10. *In vitro* procedure used for the measurement of the degree of phytate hydrolysis.

CROP

5 g feedstuff containing phytase enzyme + 25 ml distilled water

Incubate 1 h

↓

PROVENTRICULUS and GIZZARD

Add 25 ml pepsin solution (pH 2.1)

Incubate 10 min

↓

SMALL INTESTINE

Add 2.6 ml 2 M NaOH, 47.4 ml cirate buffer (pH 6.0), and 1 ml pancreatin solution

Incubate for 3 h

↓

Place samples in liquid nitrogen for 2 min

and freeze dry

↓

Analyze sample for phytate content

analyzed for phytate content and degree of phytate hydrolysis was calculated by difference between enzyme and non-enzyme treated samples.

IN VIVO STUDY

An experiment was conducted to determine the effect of dietary phytase supplementation on the performance of broiler chickens fed wheat/canola meal-based diets containing different levels of nP. One-day old vaccinated (Marek's) male broiler chicks were obtained from a commercial hatchery and housed in a Jamesway battery brooder[4] for 4 days prior to experiment. During this period, chicks were fed a commercial chick starter diet containing 20% protein. At the start of the experiment, birds were fasted for 4 h, weighed, and placed into 5 weight groups. Chicks were randomly distributed from the weight groups to 70 pens (5 chicks per pen) in electrically-heated Petersime brooders[5] in an environmentally controlled room. Chicks were provided with continuous light and were given feed in mash form and water *ad libitum*.

The chicks were randomly assigned to 7 dietary treatments (10 replicates/treatment) which contained three levels of nP and two levels of supplemental phytase. Two basal diets containing 0.25 and 0.35% nP were prepared along with a 0.45% nP diet which served as a positive control (Table 5). All diets were formulated to meet nutrient requirements, with the exception of nP, as specified by NRC (1994). A commercial phytase preparation,

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[4]James Mfg. Co., Mount Joy, PA
[5]Petersime Incubator Co., Gettysburg, OH 45328

TABLE 5. Composition (g/kg) of experimental diets.

Ingredient	Basal Diet 1	Basal Diet 2	Positive Control Diet
Canola meal	303.0	306.2	308.2
Wheat	576.1	568.3	562.4
Casein	17.0	17.0	17.0
DL-methionine	0.5	0.5	0.5
Lysine	1.5	1.5	1.5
Vegetable oil	63.0	65.0	66.5
Limestone	19.0	16.8	14.5
Biophos	1.90	6.7	11.4
Vitamin premix[a]	10.0	10.0	10.0
Mineral premix[b]	5.0	5.0	5.0
Chromic oxide	3.0	3.0	3.0
Calculated Nutrient Content[c]			
Crude protein	221	221	221
Energy (kcal/kg)	3054	3054	3054
Non-phytate P	2.5	3.5	4.5
Calcium	9.9	10.0	10.0
Lysine	11.1	11.1	11.1
DL-methionine	5.0	5.0	5.0
Met + Cys	9.7	9.7	9.7

[a]Amount supplied per kilogram diet: vitamin A, 8250 IU; vitamin D3, 1000 IU; vitamin E, 11 IU; vitamin B_{12}, 11.5 ug; vitamin K, 1.1 mg; riboflavin, 5.5 mg; Ca-pantothenate, 11 mg; niacin, 53 mg; choline chloride, 1056 mg; folic acid, .75 mg; biotin, .25 mg; ethoxyquin, 125.0 mg; methionine-D1, 500.0 mg.

[b]Amount supplied per kilogram diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.36 mg.

[c]Based on analyzed and NRC (1994) feed composition data.

Natuphos® 5000, was supplemented to the 0.25 and 0.35% nP diets providing 0, 500, and 1000 U phytase/kg diet based on the manufacturer's stated activity. The enzyme was added to experimental diets in the form of premixes which were prepared using 1 kg subsamples from the respective basal diets. Chromic oxide was used as an internal marker and a calcium level of 1% was maintained for all dietary treatments. The dietary content of phytate was similar in all diets at 0.46% since all phytate was supplied from wheat and canola meal. Diets containing the three levels of nP, 0.25, 0.35, and 0.45% were calculated to provide 0.71, 0.81, and 0.91% total P, respectively.

The chicks were fed the experimental diet for 14 days (5-19 days of age). Body weights after a 4 h fast and feed intake were measured on a pen basis on days 7 and 14 of the experiment. Records of mortality and leg problems were also maintained. On day 15 of the experiment, 15 birds per treatment were used for digestibility measurements. Three samples of excreta per treatment were collected over a 3 h period. Each sample consisted of pooled excreta from 5 birds. Excreta samples were placed in plastic bags, frozen and freeze dried. Following excreta collection the birds were killed by cervical dislocation and digesta from the terminal ileum (7.5-10.0 cm prior to ileocecal junction) were collected, frozen in liquid nitrogen, and freeze dried. Toe samples were obtained by severing the middle toe through the joint between the second and third tarsal bones from the distal end. Five toes of all chicks in a pen were pooled yielding three samples of toes per treatment. The toe samples were dried for 12 h at 100°C to a constant weight and ashed in a furnace at 600°C for 12 h. Toe ash was expressed as a percentage of dry weight.

Fourteen additional birds fed the positive control diet were used to determine pH of

the various segments of the gastrointestinal tract. Birds were killed by cervical dislocation and the gastrointestinal tract removed and split into sections. The digesta from each of the crop, proventriculus, gizzard, duodenum, jejunum, ileum, ceca, and colo-rectum of 2 birds were pooled and the pH tested with a pH meter[6]. A small amount of deionized water was added to the samples if there was insufficient amounts of aqueous material to obtain a reading.

Chemical Analysis

Diets and excreta samples were ground to pass through a 1 mm sieve whereas digesta from the terminal ileum was ground in a mortar due to small sample size. The excreta, terminal ileum digesta and feed samples were analyzed for chromic oxide (Williams et al., 1962), phytate P and total P (AOAC, 1975). The content of phytate was determined according to a modified method of Haug and Lantzsch (1983). This method is based on the precipitation of ferric phytate following addition of a standardized solution of ferric chloride in dilute hydrochloric acid. After removal of ferric phytate precipitate by centrifugation the excess of unprecipitated ferric ion is then measured colorimetrically. The decrease of iron in the supernatant is proportional to the phytate content of the sample. In brief, a 40 mg sample was accurately weighed and shaken with 10 ml 0.2 N HCl for 3 h at room temperature. After filtration, 1 ml filtrate and 2 ml ferric solution in triplicate were placed in hydrolysis tubes and boiled for 30 min. After cooling in ice water for 15 min the tubes were

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[6]Accumet pH Meter, Model 810

allowed to adjust to room temperature, after which the samples were shaken and centrifuged[7] at 3000 rpm for 30 min. One ml of supernatant was then transferred into another tube and 1.5 ml bipyridine solution (Aldrich, Canada) was added. After 10 min the absorbance was measured[8] at 519 nm against distilled water. Phytate was quantified from a standard curve developed using solutions of known amounts of phytic acid (Sigma, Canada).

Stastical analysis

Data were analyzed by the General Linear Models (GLM) procedure of the SAS Institute, Inc. (1986) with pen means as experimental units. Where significant effects were found, tests for differences between individual means were determined using the Duncan's multiple range test. The α-level for significance was $p < 0.05$.

RESULTS

IN VITRO STUDY

The results of *in vitro* hydrolysis of phytate in the diet and individual feedstuffs are shown in Table 6. After incubation of canola meal, WC, and WF with phytase, 100, 37, and 61% of phytate was hydrolyzed, respectively. No phytate could be detected in the canola meal sample remaining after the GI tract simulation indicating its full hydrolysis. Grinding

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[7]Centra GP8, International Equipment Co., Needham Heights, MA
[8]Ultrospec 2000, Pharmacia Biotech, Cambridge, England

of the wheat sample through a 1 mm sieve increased phytate hydrolysis by 65%. When WC and WF were combined with canola meal, 72 and 90% of the phytate was hydrolyzed, respectively. The hydrolysis of phytate in a wheat/canola meal diet containing 1000 units phytase/kg was much lower and averaged 46%.

IN VIVO STUDY

The effects of dietary phytase and nP levels on feed intake, body weight gain, and feed conversion of broiler chickens (5-19 days) are shown in Table 7. Birds fed 0.35% nP without phytase addition had similar body weights, feed intake, and feed conversion as birds fed the 0.45% nP diet. Consequently, phytase addition to 0.35% nP diets showed no response. However, phytase addition to the diet containing 0.25% nP significantly improved ($P<0.05$) feed intake and weight gain. Feed intake and body weight gain of birds fed 0.25% nP with 1000 units phytase/kg were similar to those of the positive control diet. Addition of 500 units phytase/kg also improved ($P<0.05$) growth rate and feed intake, however, maximum response was not attained at this level of supplementation. The efficiency of feed utilization, as measured by feed to gain ratio, was influenced by phytase addition and level of nP. Increasing levels of nP from 0.25 to 0.45% improved ($P<0.05$) feed to gain ratio. The addition of 1000 units phytase/kg to 0.25% nP resulted in feed to gain ratio comparable to positive control.

The digestibility of phytate as determined at the terminal ileum and in the excreta is presented in Table 8. Generally, the addition of phytase improved ($P<0.05$) phytate digestibility as determined at the terminal ileum. Addition of 1000 units phytase/kg to 0.25% nP and 500 units phytase/kg to 0.35% nP improved phytate digestibility by 19% compared

TABLE 6. Phytate content and enzymatic hydrolysis of phytate in canola meal, wheat, and a wheat/canola meal diet.

	Phytate content (%)	Phytase (U/kg)	Phytate hydrolyzed (% of original)	Std. dev
Canola meal (CM)	3.0	1850	100	1.5
WC[1]	1.4	900	37	1.1
WF[2]	1.4	900	61	2.4
WC/CM[3]	1.9	1100	72	1.8
WF/CM[3]	1.9	1100	90	2.2
Wheat-CM diet	1.7	1000	46	1.5

[1]Wheat - ground in hammer mill (coarse)
[2]Wheat - ground to pass through 1 mm sieve (fine)
[3]66% wheat - 34% canola meal
[4]Basal diet 1 (see Table 5 for details)

TABLE 7. Feed intake, body weight gain, and feed:gain ratios of broilers fed wheat/canola meal diets containing varying levels of non-phytate phosphorus and supplemental phytase from 5 to 19 d of age

nP (%)	Phytase added (U/kg)	Feed intake (g/bird)	Weight gain (g/bird)	Feed:Gain
0.25	0	565[c]	373[c]	1.52[a]
0.25	500	607[b]	399[b]	1.52[a]
0.25	1000	627[ab]	419[a]	1.50[ab]
0.35	0	638[a]	427[a]	1.49[ab]
0.35	500	640[a]	437[a]	1.47[b]
0.35	1000	635[a]	429[a]	1.48[ab]
0.45	0	641[a]	435[a]	1.47[b]
Pr.>F		.0001	.0001	.0436
SEM		6.4	8.2	.01

[abc]Means in the same column with different letters differ significantly ($P<0.05$)

to diets without phytase. The digestibility of phytate, as measured in the excreta, was not influenced by dietary phytase and nP. Unexpectedly, terminal ileum phytate digestibility was similar or slightly higher than that determined in the excreta. The influence of phytase and level of nP on ash percentage of dried toes tended to parallel that of feed intake and body weight gains (Table 8). At the low level of nP (0.25%), toe ash percentage was improved ($P<0.05$) by the addition of phytase, however, the magnitude of response did not equal that of the 0.35% nP or 0.45% nP positive control diet. As shown in Table 8, the amount of P excreted (g/kg DM) was largely influenced by nP. The lowering of nP to 0.35% and 0.25% reduced P excretion by 24% and 29%, respectively. No response to phytase supplementation was observed at 0.35% nP, however, addition of 500 and 1000 units phytase/kg to 0.25% nP reduced P excretion by 38% compared to that of the positive control diet.

The pH of digesta in various sections of the gastrointestinal tract are presented in Table 9. The pH of digesta ranged from 2.5 in the proventriculus to 6.8 in the ileum. The pH of the digesta in the gizzard was 2.8 and increased to 5.8 upon reaching the duodenum and jejunum. The digesta in the caeca was slightly more acidic (pH 5.6) than the digesta in the ileum.

DISCUSSION

IN VITRO STUDY

After incubation of canola meal with 1850 units phytase/kg, no phytate could be detected in the sample indicating complete phytate hydrolysis. In contrast, incubation of WC

TABLE 8. Phytate digestibility, ash percentage of dried toes, and phosphorus excretion of broilers fed wheat/canola meal diets containing varying levels of non-phytate phosphorus and supplemental phytase from 5 to 19 d of age

nP (%)	Phytase added (U/kg)	Phytate digestibility[1] (%)	Phytate digestibility[2] (%)	Toe ash (%)	P excretion (g/kg DM)
0.25	0	52.1[b]	48.2	6.9[c]	11.6[c]
0.25	500	56.3[ab]	53.7	8.7[b]	10.3[d]
0.25	1000	61.6[a]	56.5	9.4[b]	10.2[d]
0.35	0	52.2[b]	47.3	10.5[a]	12.5[bc]
0.35	500	62.4[a]	48.6	11.3[a]	12.9[bc]
0.35	1000	59.0[ab]	52.2	11.6[a]	13.1[b]
0.45	0	53.5[b]	49.4	11.5[a]	16.4[a]
Pr.>F		.0191	.2956	.0001	.0001
SEM		2.2	2.9	.38	.04

[abcd]Means in the same column with different letters differ significantly (P<0.05)
[1]as determined at the terminal ileum
[2]as determined in the excreta

TABLE 9. pH of digesta in various sections of the gastrointestinal tract of broilers

	pH
Crop	4.5∓0.2
Proventriculus	2.5∓0.3
Gizzard	2.8∓0.4
Duodenum	5.8∓0.3
Jejunum	5.8∓0.2
Ileum	6.8∓0.3
Caeca	5.6∓0.3
Colon	6.3∓0.4

and WF with 900 units phytase/kg yielded 37 and 61% hydrolysis, respectively. The canola meal phytate was more readily and extensively hydrolyzed than the wheat phytate. This may be due to the location of phytate and the processing of these feedstuffs. Canola phytate is found in the crystalline globoids inside protein bodies in the cell of the radicle and cotyledon (Yiu et al., 1982). Pressing, solvent extraction, and heat treatment are involved in the processing of canola into oil and meal. These chemical and physical processes may disrupt the radicle and cotyledon making the phytate substrate more available. Han and Wilfred (1988) applied a crude phytase enzyme to soybean and cottonseed meals and studied the factors affecting hydrolysis of phytate. After a 5 h incubation at pH 5.4, about 85% of the phytate in SBM was hydrolyzed by the microbial phytase whereas only 67% of the phytate in cottonseed meal was destroyed by the same enzyme treatment. The researchers proposed that since soybean phytate was uniformly distributed in the aleurone grains it was more readily hydrolyzed, whereas, cottonseed phytate is segragated in small globoid deposits which makes access of the enzyme to the substrate difficult. Earlier studies from this laboratory (Guenter et al., 1995) documented that the degree of phytate hydrolysis on incubation of canola meal with 1000 units/kg of phytase at 40°C and pH 5.4 for 60 min was minimal (ie., 13% of total) and an eight fold increase in enzyme concentration resulted in only a slight increase in phytate hydrolysis (ie., 17%). It was confirmed in a study by Newkirk (1996) in which a crude phytase preparation did not effectively hydrolyse phytate in canola meal at pH 5.8. After a 23 h incubation period, total phytate hydrolysis was finally achieved. These studies indicate that canola phytate is linked to other components of the meal (ie., divalent cations, protein, etc.) which reduces its solubility and thus accessibility of phytase to the substrate at pH 5-6.

In the current experiment, the use of pepsin-HCl solution (pH 2.1) may have aided in dissociating phytate from the protein bodies of canola meal, providing a readily available substrate for the enzyme to hydrolyze. The results are in agreement with Zyla and Koreleski (1993) in which rapeseed meal was subjected to phytate hydrolysis using enzyme preparations containing phytase and acid phosphatase activities. They obtained complete conversion of myo-inositol hexaphosphate to lower phosphate esters of myo-inositol in rapeseed meal after a 4 h incubation at 40°C and pH 4.5.

Under commercial conditions wheat undergoes very little processing. Coarse grinding is a common practice and as such leaves a physical barrier for the phytase enzyme to access the substrate. In this context, wheat phytate is largely found (80%) in the aleurone layer (O'Dell et al., 1972) and this layer may remain undisturbed after grinding through a conventional hammermill. This may be proven by the results of the current study in which grinding the wheat to pass through a 1 mm sieve resulted in a 65% improvement (37 vs. 61%) in phytate hydrolysis. On the other hand, grinding the wheat may have also increased endogenous phytase activity. In the *in vitro* study by Nys et al. (1996) it was shown that the degree of grinding influenced phytase activity with higher activity being found in finer ground (0.5 to 1 mm) wheat samples. It seems obvious that the substrate and the enzyme are brought together in the grinding process resulting in improved phytate hydrolysis.

Combining wheat with canola meal at the ratio 2:1 (w/w) would result in a total phytate content of 1.9%. Using the phytate content values from Table 6, in a 5 g sample of this mixture there would be 97.2 mg of phytate, of which 46.2 mg would be contributed by wheat phytate and 51.0 mg by canola phytate. It appears from the *in vitro* studies that canola

phytate was completely hydrolyzed in the wheat/canola mixture, whereas, the wheat phytate followed a similar incomplete hydrolysis pattern as that when wheat was incubated alone. In this regard, the phytate hydrolysis of the WC- and WF-canola meal mixture was 72% and 90 %, respectively. However, upon incubation of a wheat/canola meal-based diet with 1000 units phytase/kg only 46% of the phytate was hydrolyzed, a value much lower than the 72% that would be expected since the phytate in the diet originated from similar sources (ie., coarsely ground wheat and canola meal). This is in agreement with studies by Zyla et al. (1995) who found that phytase enzyme was not able to completely dephosphorylate phytates in corn-soybean feed under simulated conditions of the gastrointestinal tract of the turkey. This indicates that there are certain components in a poultry diet which affect phytate hydrolysis. One component which has been shown to influence phytate hydrolysis is calcium (Ballam et al, 1985). Generally, poultry diets are high in Ca and the NRC (1994) recommended Ca level is 1% for broilers and 3-4% for laying hens. At these high Ca levels an insoluble Ca-phytate complex may be formed. In addition the high Ca may repress phytase activity by competing for the active sites of the enzyme (Wise, 1983). It may be hypothesized that improved hydrolysis of phytate will occur if lower Ca levels are used in poultry diets. Other components found in poultry diets such as inorganic P, minerals, and synthetic amino acids may also adversely affect phytate hydrolysis through formation of insoluble phytate complexes or inhibiting phytase activity.

IN VIVO STUDY

The results demonstrate that supplemental phytase is effective in improving P

availability in wheat/canola meal diets. The magnitude of response to phytase supplementation was related to the level of nP with the greatest response occurring at low nP levels. The addition of 500 and 1000 units phytase/kg to diets containing 0.25% nP resulted in increased body weight gain by 7 and 12%, respectively. In addition, the body weight gain of birds fed 0.25% nP diet supplemented with 1000 units phytase/kg was comparable to that of the positive control diet (0.45% nP). Similarly to body weight gain, feed intake increased by 7 and 11% following addition of 500 and 1000 units phytase/kg, respectively. Since the increase in body weight gain was a direct consequence of increased feed intake, only moderate improvements in feed to gain ratio were noted. This finding is in agreement with the results reported for broiler chickens fed P-deficient corn/SBM diets (Kornegay et al., 1996).

As evidenced by chemical analyses, the enhancement of chick performance was related to improved utilization of dietary P. This was demonstrated by increased toe ash percentage, improved phytate digestibility, and reduced P excretion. The addition of 500 and 1000 units phytase/kg to diets containing 0.25% nP resulted in a 26 and 36% increase in toe ash percentage, respectively. This is in agreement with studies by Denbow et al. (1995) who demonstrated that supplementation of 1000 units phytase/kg to a P-deficient broiler chicken diet containing 0.20% nP increased toe ash percentage from 8.2% to 11.1%. In the experiment described herein, toe ash percentage was increased through phytase supplementation of the 0.25% nP diet, however, it (9.4%) did not reach the level (10.5%) of the positive control diet. If the study had carried on past 19 days we may suspect, based on toe ash percentage, that birds on these diets would have developed leg problems.

Phosphorus excretion (g/kg DM) decreased linearly with reduced dietary P content. By reducing the nP content to 0.35%, P excretion decreased by 24%. Supplementation of 1000 units phytase/kg to a 0.25% nP diet further reduced P excretion by 37%. This is in agreement with previous findings by Kornegay et al. (1996) who found that in comparison with a positive control diet (4.5 g nP/kg), P excretion was reduced by 25-54% with the addition of 200-1200 units phytase/kg at various nP levels, with the magnitude of response being greatest at low nP levels (2.0 and 2.7 g nP/kg). In the U.S. it is estimated that 250 000 tons of P are produced annually as a waste product by poultry (Cromwell and Coffey, 1991). As an example, a 37% reduction in P excretion would equate to 92 500 tons less P excreted annually in the U.S. This clearly indicates that the addition of phytase to poultry diets may be an effective means of reducing environmental pollution.

In our study, the effect of supplemental phytase on digestibility of phytate P was measured at the terminal ileum and in the excreta by the chromic oxide marker method. Digestibility at the terminal ileum was chosen to eliminate the effect of phytate hydrolysis by bacterial microflora. It was found that approximately 52-53% of the phytate from unsupplemented diets could be hydrolyzed to the end of the small intestine. This is in contrast to the findings of Nelson (1976) and Matyka et al. (1990) in which phytate P retention in chicks fed corn/SBM diets was found to be 13-17%. The inclusion of wheat in the diets may account for the high percentage of phytate digestibility. It is known that wheat P is much more available than corn P due to the presence of active endogenous phytases in wheat which may act on dietary phytate and improve overall P availability (Pointillart et al., 1984). Heat-treatment of the wheat used in the current study could have been applied to eliminate any

effect of endogenous plant phytase. Phytate digestibility may also be attributable to the chicks' inherent ability to hydrolyze dietary phytate by intestinal phytase. Early studies concluded that phytase activity in chick intestinal mucosa was minimal and was due to non-specific phosphatases (Maddaiah et al., 1964a). More recently, Maenz et al. (1997) found that the small intestinal brush border membrane of the chicken contains a phytase activity that is distinct from non-specific acid and alkaline phosphatases. Phytase activity of broiler chicks and laying hens was highest in the duodenum (24 nmoles P hydrolyzed/mg brush border protein/min) and lowest in the ileum (14 nmoles P hydrolyzed/mg/min). The authors concluded that endogenous brush border phytase may contribute to the digestibility of dietary phytate. The results described herein appear to prove that chicks are able to digest a good portion of dietary phytate naturally.

Supplementation of phytase indicated a positive influence on phytate digestibility as determined at the terminal ileum. The addition of 1000 units phytase/kg to 0.25% nP resulted in a 19% increase in phytate digestibility as compared to unsupplemented diets. The hydrolysis of phytate yielded phosphate and possibly Ca which were subsequently utilized by the chickens as evidenced by increased weight gain and toe ash percentage. A less pronounced response to phytase supplementation was observed when phytate digestibility was determined in the excreta samples.

In general, it was thought that phytate digestibility would be slightly higher in excreta than terminal ileum due to phytate hydrolysis by microflora of the lower gut. In fact, phytate digestibility as determined in the excreta was similar to or lower than that determined in the terminal ileum. Lower digestibility of phytate may be explained by phytate synthesis

occurring in the ceca and colon or during the period of excreta collection. Earlier findings have indicated that phytate synthesis may occur in fecal samples collected from chicks fed a phytate-free, casein-gelatin based diet when kept under conventional conditions (Savage et al., 1964). When similar chicks were maintained in a germ-free environment, however, the feces contained no phytate. Jenkins (1965) found that microorganisms in poultry feces were capable of phytate synthesis if there was an appreciable delay between the time of collection of poultry droppings and their analysis. Since in the current experiment the collection period was 3 h, after which time the samples were placed in a freezer, it is questionable if there was sufficient time or conditions for phytate synthesis. More recently, Sooncharernying and Edwards (1993) determined that holding wet fecal samples at room temperature (22°C) from 0 to 32 h before analysis for inositol phosphates had no effect on the concentration of inositol hexaphosphate. Their results indicated very little hydrolysis or synthesis of inositol phosphate in the excreta of chickens as they lay on a dropping tray.

It is important to have an accurate account of the nP requirement for poultry when formulating diets supplemented with phytase enzyme. Feeding a diet containing sufficient amount of available P would result in no response to phytase supplementation. In a study conducted by Boling et al. (1997), laying hens were fed corn/SBM-based diets containing 0.10, 0.15, 0.20, 0.25 and 0.45% nP. Phytase was added to the three lowest nP diets at a level of 300 units per kg of diet. Birds fed the 0.10% nP diet with no supplemental phytase resulted in significantly lower egg production and egg yield, feed consumption, feed efficiency, body weight and increased mortality compared to other dietary treatments. No differences in growth and production were observed for diets containing 0.15, 0.20, 0.25, and

0.45% nP. This indicates that the nP requirement of laying hens is approximately 0.15%, consequently, phytase addition to 0.15 and 0.20% nP showed no response. The addition of 300 units phytase/kg to the 0.10% nP diet restored production and performance to levels obtained with 0.15% nP or higher. According to the experiment described herein, the nP requirement of broiler chickens from 0 to 3 weeks old appears to be 0.35%. No differences in weight gain, feed intake, and toe ash percentage were observed between 0.35% and 0.45% nP, thus, the NRC (1994) recommendation of 0.45% may be overestimated for wheat/canola meal diets. It may also indicate that the nP requirement of the broiler chicken changes as the bird grows older. A nP level of 0.45% may be the requirement for the first week of growth, but then decreases to 0.35% for the remainder of the growth period.

The high level of Ca used in the current study may have reduced the overall response to supplemental phytase and inorganic P level. It has been shown that dietary Ca level and Ca to total P ratio affects phytate hydrolysis in chickens (Ballam et al., 1985), pigs (Sandberg et al., 1993), and rats (Nahepetian and Young, 1980). At high levels of dietary Ca, an insoluble Ca-phytate complex may form which is poorly available to enzymatic hydrolysis (Wise, 1983). A study with young turkeys conducted by Qian et al. (1996a) demonstrated that widening the Ca to tP ratio produced detrimental effects on BW gain, feed intake, gain:feed, toe ash percentage, and apparent utilization of Ca and P. Widening the Ca to tP ratio from 1.4 to 2.0:1 decreased phytase efficacy by 7.4 and 4.9%, respectively, for 0.27 and 0.36% nP diets. The best responses to supplemental phytase were achieved when poults were fed diets with Ca to tP ratios ranging from 1.1 to 1.4:1. In the present study, Ca to tP ratios were 1.1, 1.2, and 1.4:1 for .45, .35, and 0.25% nP diets, respectively. The Ca to tP ratios

used were generally quite low due to the high level of phytate, however, it may be the level of Ca which is more critical to examine.

The pH of digesta in various sections of the GI tract were examined to determine if conditions were suitable for phytase enzyme activity. The pH profile of microbial phytase from Natuphos® has two pH optima at pH 2.5 and pH 5.5 (Heinzl, 1996). The pH of digesta in the crop, proventriculus, gizzard, duodenum, and jejunum were found to be 4.5, 2.5, 2.8, 5.8, and 5.8, respectively. All these pH values fall within the relative phytase activity (>50%) of Natuphos® , consequently, phytase should remain active in the upper GI tract. As digesta moves into the ileum the pH increases to 6.8 where phytase activity would be minimal. In a study by Kornegay and Yi (1996), phytase levels ranging from 500 to 1000 units/kg were included in diets of chickens. Most of the phytase activity was observed in the crop (69 to 86% of diet phytase activity) with about a 50% reduction in the proventriculus. No phytase activity was detected in the small intestine which may indicate that phytase is broken down by proteases in the small intestine, thus, making it undetectable. Although the pH conditions of the upper GI tract appear to be favourable for phytase activity, it is possible that the enzyme is to some extent denatured in the proventriculus and gizzard subsequently affecting phytate hydrolysis in the small intestine.

CHAPTER 4

THE EFFECT OF VARYING CALCIUM AND CHOLECALCIFEROL LEVELS ON PHYTATE DIGESTIBILITY AND PHOSPHORUS UTILIZATION IN BROILER CHICKENS FED WHEAT/CANOLA MEAL DIETS SUPPLEMENTED WITH PHYTASE ENZYME

ABSTRACT

In vitro and *in vivo* experiments were conducted to determine the effect of Ca on phytate hydrolysis in canola meal and wheat/canola meal diets supplemented with phytase enzyme. The *in vitro* experiment was designed to simulate the intestinal conditions of the chicken and the extent of phytate hydrolysis was determined from phytate disappearance during incubation. Treatments were set up in a 4x5 factorial arrangement in which canola meal was supplemented with calcium carbonate to provide five levels of Ca (0.65, 0.80, 1.2, 1.6, 2.0%) and four levels of phytase (0, 1000, 2000, 3000 units/kg). Complete hydrolysis of phytate was attained at the 0.65 and 0.80% Ca level with 2000 and 3000 units phytase/kg of canola meal. Phytate hydrolysis decreased linearly with increasing levels of Ca at all levels of phytase supplementation. A greater degree of phytate hydrolysis was observed with addition of 3000 units/kg phytase and at low Ca levels. An *in vivo* study set up in a 2x2x2 factorial arrangement was conducted to determine the effect of Ca, cholecalciferol, and phytase on phytate P utilization in broiler chickens. A low P (0.25% nP) wheat/canola meal diet was supplemented with two levels of Ca (0.7 and 0.8%); cholecalciferol (1000 and 5000 IU); and a commercial phytase preparation, Natuphos® 5000 (0 and 1000 units/kg). A positive control diet contained 0.45% nP and 1% Ca. In comparison to the positive control,

no differences in feed intake, weight gain, and feed:gain were observed for the 0.7% Ca diets regardless of enzyme supplementation. A negative response was noted for diets containing 0.8% Ca which was overcome by phytase supplementation. The addition of phytase improved ($P<.05$) phytate and Ca digestibility with maximum digestibility obtained with both phytase and cholecalciferol supplementation at the 0.8% Ca level. The addition of cholecalciferol alone had no effect on parameters measured. Lowering of Ca and nP levels had an adverse effect on toe ash percentage. Phytase and cholecalciferol supplementation to 0.8% Ca provided a toe ash percentage similar to that of the positive control. The addition of phytase to low nP diets reduced P excretion (g/kg DM) by 35%. The results of the study show that phytase supplementation to low P wheat/canola meal diets improves phytate P utilization and that the level of Ca in the diet is a critical factor influencing phytate digestibility.

Key words: phytate, phosphorus, calcium, cholecalciferol, phytase, broiler chicken

INTRODUCTION

The use of supplemental phytase in poultry diets has proven to be an effective means of improving phytate P availability and decreasing P excretion (Simons et al., 1990; Kornegay et al., 1996). Yi et al. (1996c) reported that the addition of 1050 units phytase/kg to a low P corn/SBM diet increased growth performance, toe ash percentage, and apparent P and Ca retention.

In much of the research with microbial phytase, only single dietary levels of Ca and vitamin D_3, both known to influence phytate P utilization in poultry (Scheidler and Sell, 1987; Edwards, 1993), were used. A high level of dietary Ca has been shown to reduce the availability of phytate P in poultry (Ballam et al., 1985), rats (Nahapetian and Young, 1980), and pigs (Sandberg et al., 1993). Calcium, a dominant divalent cation in animal diets, has the ability to form an insoluble complex with phytate (Wise, 1983), rendering phytate P and Ca unavailable for intestinal absorption.

Mohammed et al. (1991) found that diets containing low levels of Ca and elevated levels of cholecalciferol allowed for greater utilization of phytate P and reduced requirement for inorganic P. In a recent study Qian et al. (1996a) reported that phytase supplementation was negatively influenced by wide Ca to tP ratios in turkey diets. In a study with broiler chickens, Sebastian et al. (1996b) showed that optimum growth performance and mineral utilization was achieved in phytase supplemented diets with a low (0.6%) level of Ca.

Since much of the research on microbial phytase has been conducted with corn/SBM diets it is difficult to predict the interaction between Ca and phytate in canola meal. Canola

meal contains a high level of phytate (2.9-3.2%, Zhou et al., 1990), therefore, it may interact with minerals such as Ca to a much greater extent than other feedstuffs. In our earlier *in vitro* study it has been shown that complete hydrolysis of canola phytate is possible with phytase addition. The primary objective of the current study was to examine the effect of Ca and phytase supplementation on hydrolysis of canola meal phytate under *in vitro* (simulated GI tract) and *in vivo* conditions. A second objective was to study the effect of lowering Ca and increasing cholecalciferol levels on broiler chicken performance when fed a low P wheat/canola meal diet supplemented with phytase enzyme.

MATERIALS AND METHODS

IN VITRO STUDY

An *in vitro* procedure was used to determine the effect of Ca on phytate hydrolysis. Canola meal with 5 graded levels of Ca (.65, .80. 1.2, 1.6, and 2.0%) each supplemented with a commercial phytase preparation, Natuphos® 5000, at 0, 1000, 2000, and 3000 units/kg were used. The content of Ca in canola meal was determined and found to be .65%. Calcium carbonate was added to treatments to provide the desired Ca level. A 5 g sample of canola meal was subjected to incubation with an appropriate phytase level under conditions simulating the gastrointestinal tract of the chicken (see Chapter 3, Figure 10). After the incubation period, samples were placed in liquid nitrogen to stop enzyme activity and were then freeze dried. The samples were analyzed for phytate content (Haug and Lantzsch, 1983) and the degree of phytate hydrolysis was calculated from its disappearance after

incubation.

IN VIVO STUDY

An experiment was conducted to determine the effect of reduced calcium and elevated cholecalciferol levels on performance of broiler chickens fed phytase supplemented wheat/canola meal diets. One-day old vaccinated (Marek's) male broiler chicks were obtained from a commercial hatchery and housed in a Jamesway battery brooder[1] for 4 days prior to the start of the experiment. During this period, chicks were fed a commercial chick starter diet containing 20% protein. At the start of the experiment, birds were fasted for 4 h, weighed, and placed into 5 weight groups. Chicks were randomly distributed from the weight groups to 81 pens (5 chicks per pen) in electrically-heated Petersime brooders[2] in an environmentally controlled room. Chicks were provided with continuous light and were given feed in mash form and water *ad libitum*.

The chicks were randomly assigned to 9 dietary treatments (9 replicates/treatment). One diet served as a positive control and contained 0.45% nP and 1000 IU cholecalciferol in accordance with NRC (1994) requirements. The remaining 8 treatments contained 0.25% nP and were organized as a 2x2x2 factorial arrangement with Ca (0.70 or 0.80%), cholecalciferol (1000 or 5000 IU), and phytase (0 or 1000 units/kg) being the variables. Two basal diets were initially mixed to provide 0.25% nP and either 0.70 or 0.80%Ca (Table 10). Phytase and cholecalciferol were added to diets in the form of premixes which

[1]James Mfg. Co., Mount Joy, PA
[2]Petersime Incubator Co., Gettysburg, OH 45328

TABLE 10. Composition (g/kg) and calculated nutrient content of diets.

Ingredient	Basal Diet 1	Basal Diet 2	Positive control Diet
Canola meal	321.6	320.0	318.0
Wheat	565.3	563.2	549.0
Casein	19.0	19.5	22.0
DL-methionine	0.3	0.3	0.3
Lysine	1.2	1.2	1.2
Vegetable oil	62.0	62.5	66.0
Limestone	10.7	13.4	14.1
Biophos	1.9	1.9	11.4
Vitamin premix[a]	10.0	10.0	10.0
Mineral premix[b]	5.0	5.0	5.0
Chromic oxide	3.0	3.0	3.0
Caculated Nutrient Content[c]			
Crude protein	219	219	219
Energy (kcal/kg)	3054	3051	3045
Non-phytate P	2.5	2.5	4.5
Calcium	7.0	8.0	10.0
Lysine	10.9	11.0	11.0
DL-methionine	4.9	4.9	4.9
Met + Cys	9.7	9.7	9.7

[a]Amount supplied per kilogram diet: vitamin A, 8250 IU; vitamin D_3, 1000 IU; vitamin E, 11 IU; vitamin B_{12}, 11.5ug; vitamin K, 1.1 mg; riboflavin, 5.5 mg; Ca-pantothenate, 11 mg; niacin, 53 mg; choline chloride, 1056 mg; folic acid, .75 mg; biotin, .25 mg; ethoxyquin, 125.0 mg; methionine-D1, 500.0 mg.

[b]Amount supplied per kilogram diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.36 mg.

[c]Based on analyzed and NRC (1994) feed composition data.

were prepared with a 1 kg subsample from the respective basal diets. Chromic oxide was added at 0.3% as an internal marker. The dietary phytate content in basal diet 1, 2, and the positive control diet was analyzed and found to be 0.39, 0.37, and 0.35%, respectively. Diets providing the two levels of nP, 0.25 and 0.45%, were also analyzed and found to contain 0.66 and 0.86% total P (tP), respectively. The chicks were fed the experimental diet for 14 days (5-19 days of age). Body weights after a 4 h fast and feed intake were measured on a pen basis on days 7 and 14 of the experiment. On day 15, samples of excreta from all pens were collected over a 12 h period. The excreta were placed in plastic bags, frozen, and freeze dried. After excreta collection, 15 birds per treatment were killed by cervical dislocation and digesta samples from the terminal ileum (7.5-10.0 cm prior to ileocecal junction) were obtained. Three samples per treatment, each consisting of digesta from 5 birds, were collected in plastic containers, frozen in liquid nitrogen, and freeze dried[3]. Toe samples were obtained by severing the middle toe through the joint between the second and third tarsal bones from the distal end. Five toes of all chicks in a pen were pooled yielding three samples of toes per treatment. The toe samples were dried for 16 h at 100°C to a constant weight and ashed in a furnace at 600°C for 12 h. Toe ash was expressed as a percentage of dry weight.

Chemical Analysis

Diets and excreta samples were ground in a coffee grinder whereas digesta from the terminal ileum was ground in a mortar due to small sample size. A 10 g sample of ground

[3]Virtis, 815 Route 208, Gardiner, NY 12525-9989

excreta from 3 pens of the same treatment was pooled to provide 3 samples per treatment. The diets, excreta, and digesta were analyzed for chromic oxide (Williams et al., 1962) and total P and Ca according to procedures of the Association of Official Analytical Chemists (AOAC, 1975). The content of phytate was determined according to a modified method of Haug and Lantzsch (1983) as in previous work. A sample of each dietary treatment was sent to the manufacturer (BASF, 363 Maxwell Crescent, Regina, SK S4N 5X9) for phytase analysis.

Stastical Analysis

Data were analyzed by the General Linear Models (GLM) procedure of the SAS Institute, Inc. (1986) with pen means as experimental units. Where significant effects were found, tests for differences between individual means were determined using the Duncan's multiple range test. The α-level for significance was $p<0.05$.

RESULTS

IN VITRO STUDY

The degree of canola phytate hydrolysis at various Ca to phytic acid (PA) molar ratios and phytase levels is shown in Figure 11. Since canola meal was found to contain 2.9% PA and 0.65% Ca, the molar ratio of 4 represented the proportion naturally occuring in the meal. Subsequent addition of Ca to 0.8, 1.2, 1.6 and 2.0% of the meal resulted in Ca to PA ratios of 5, 7.5, 10, and 12.5, respectively. Phytate hydrolysis with no added phytase averaged

14.8% and was independent of Ca level. However, 67% of phytate was hydrolyzed when 1000 units of phytase per kg of canola meal was applied. Addition of 2000 or 3000 units of this enzyme resulted in complete hydrolysis of canola phytate. In general, as the level of Ca and/or Ca:PA molar ratio increased, the amount of phytate hydrolyzed decreased. The lowest degree of phytate hydrolysis occured at 2% Ca and/or 12.5 Ca to PA molar ratio, regardless of supplemental phytase level. As compared to phytate hydrolysis at Ca:PA molar ratio of 4, the highest Ca level (ie., Ca:PA molar ratio of 12.5) resulted in reduced phytate hydrolysis by 42, 48, and 11%, respectively, for treatments containing 1000, 2000, and 3000 phytase units/kg.

IN VIVO STUDY

The effect of phytase supplementation on feed intake, weight gain, and feed conversion of broilers fed different levels of Ca and cholecalciferol is shown in Table 11. Main effects and interactions of phytase, calcium, and cholecalciferol are presented in Appendix Table 1. Birds fed 0.25% nP and 0.7% Ca had similar body weight gain and feed intake as birds fed the 0.45% nP and 1% Ca diet, consequently, the addition of phytase and cholecalciferol showed no response. However, phytase supplementation to the 0.8% Ca diet increased ($P<0.05$) feed intake and weight gain. The addition of both cholecalciferol and phytase to this diet further improved ($P<0.05$) feed intake. The efficiency of feed utilization, as measured by feed to gain ratio, was not influenced by phytase and cholecalciferol addition.

The effects of Ca level and phytase and cholecalciferol supplementation on phytate and calcium digestibility are presented in Table 12. See Appendix Table 2 for main effects

FIGURE 11. Effect of calcium and phytase supplementation on canola phytate hydrolysis *in vitro*

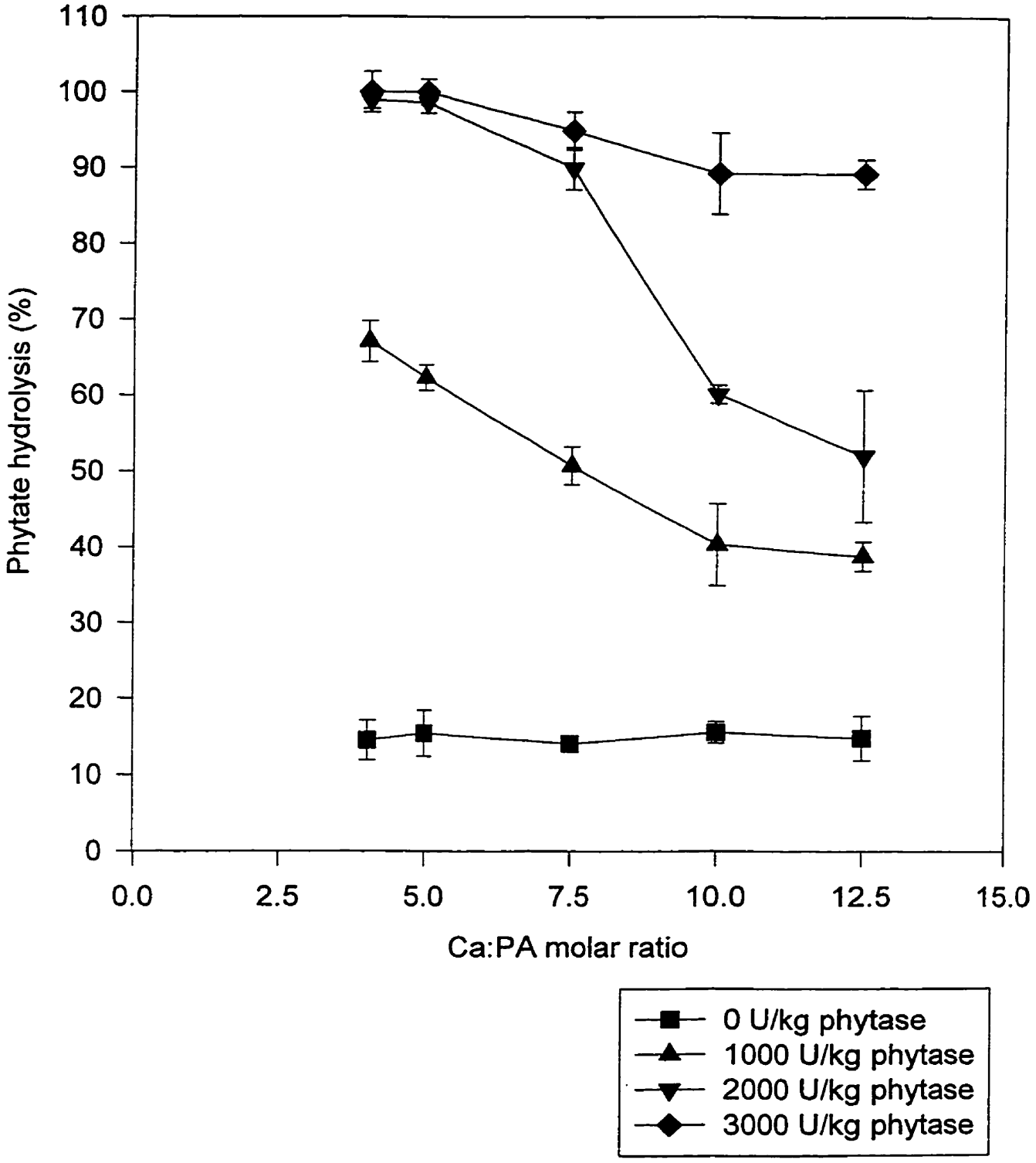

TABLE 11. Feed intake, body weight gain and feed:gain ratios of broilers fed wheat/canola meal diets containing varying levels of calcium, cholecalciferol (D_3), and supplemental phytase from 5 to 19 d of age

nP (%)	Ca (%)	D_3 (IU)	Phytase added (U/kg)	Feed intake (g/bird)	Weight gain (g/bird)	Feed:Gain
0.25	0.7	1000	0	640[a]	420[a]	1.52[a]
0.25	0.7	5000	0	632[ab]	416[a]	1.52[a]
0.25	0.7	1000	1000	630[ab]	420[a]	1.50[ab]
0.25	0.7	5000	1000	647[a]	430[a]	1.50[ab]
0.25	0.8	1000	0	595[c]	395[b]	1.51[ab]
0.25	0.8	5000	0	599[c]	395[b]	1.52[a]
0.25	0.8	1000	1000	614[bc]	415[a]	1.48[b]
0.25	0.8	5000	1000	641[a]	427[a]	1.50[ab]
0.45	1.0	1000	0	645[a]	428[a]	1.50[ab]
SEM				7.5	5.9	0.01

[abc]Means in the same column with different letters differ significantly ($P<.05$)

and factorial analysis. The digestibility of phytate as determined at the terminal ileum and apparent phytate digestibility from the excreta followed similar patterns. The phytate digestibility as determined in the excreta was higher by 10-15 percentage points compared to terminal ileum phytate digestibility There was a significant effect of Ca on phytate digestibility with the lowest phytate digestibility found in diets containing 0.45% nP and 1% Ca. The lowering of the level of Ca and nP in the diet generally improved ($P<0.05$) phytate digestibility by 4-10 percentage points. Phytase supplementation to both 0.7 and 0.8% Ca diets increased ($P<0.05$) phytate digestibility. The addition of 1000 phytase units/kg to low Ca diets improved phytate digestibility by approximately 25% compared to low Ca diets without phytase. No effect of cholecalciferol on phytate digestibility was observed except in the diet containing 0.8% Ca supplemented with phytase and cholecalciferol. The apparent phytate digestibility in this diet was further improved ($P<0.05$) through supplementation of both phytase and cholecalciferol as compared to phytase alone. The reduction of nP and Ca showed no effect on Ca digestibility. Calcium digestibility averaged 51% in diets containing no supplemental phytase and cholecalciferol. The addition of 1000 units/kg phytase improved ($P<0.05$) Ca digestibility by 15-20% compared to unsupplemented diets. Supplementation of cholecalciferol showed no improvement in Ca digestibility.

The toe ash percentage and P excretion of broilers fed the various dietary treatments are presented in Table 13. Appendix Table 3 presents main effects of calcium, phytase, cholecalciferol. Reducing nP and Ca decreased toe ash percentage ($P<0.05$) with the lowest toe ash percentage in birds fed 0.25% nP and 0.7% Ca. The addition of phytase improved ($P<.05$) toe ash percentage of broilers fed 0.7% Ca to a level similar to that of 0.25% nP and

TABLE 12. Effect of dietary calcium, cholecalciferol (D_3), and supplemental phytase on phytate and calcium digestibility of broilers fed wheat/canola meal-based diets

nP (%)	Ca (%)	D_3 (IU)	Phytase added (U/kg)	Phytate dig.[1] (%)	Phytate dig.[2] (%)	Calcium dig.[2] (%)
0.25	0.7	1000	0	39.4^{de}	51.9^{d}	52.0^{c}
0.25	0.7	5000	0	42.5^{cd}	57.1^{cd}	54.0^{bc}
0.25	0.7	1000	1000	48.3^{bc}	67.2^{a}	60.6^{ab}
0.25	0.7	5000	1000	49.2^{abc}	65.7^{ab}	59.6^{ab}
0.25	0.8	1000	0	43.0^{cd}	53.0^{d}	49.2^{c}
0.25	0.8	5000	0	46.3^{c}	55.1^{d}	52.4^{c}
0.25	0.8	1000	1000	53.3^{ab}	61.6^{bc}	59.3^{ab}
0.25	0.8	5000	1000	55.4^{a}	67.9^{a}	61.2^{a}
0.45	1.0	1000	0	35.7^{e}	43.4^{e}	52.3^{c}
SEM				2.1	1.7	2.1

[a-c]Means in the same column with different letters differ significantly ($P<.05$)

[1] as determined at the terminal ileum

[2] as determined in the excreta

0.8% Ca. The maximum toe ash percentage was observed at 0.45% nP and 1% Ca. The addition of phytase and cholecalciferol showed a slight response at 0.8% Ca. Phytase and cholecalciferol supplementation of the 0.8% Ca diet provided a toe ash percentage similar to that of the positive control. By reducing nP from 0.45 to 0.25%, a 25% decrease (11.1 vs. 14.9 g/kg DM) in P excretion was observed. Phytase supplementation of low nP diets further decreased ($P<0.05$) P excretion by 35% as compared to positive control.

A sample of each dietary treatment was analyzed for phytase activity. The results of the analysis along with calculated phytase activity is shown in Table 14. Dietary treatments which contained no added exogenous phytase were shown to have fairly high levels of phytase activity which can be attributed to endogenous enzyme of plant origin. Approximately 550 to 600 units/kg of phytase activity was contributed by wheat, consequently, the addition of 1000 units/kg exogenous phytase resulted in a phytase activity somewhat higher (1600 units/kg) than the calculated value. It appears that the amount of enzyme added is quite accurate and mixing was adequate to obtain a homogenous and high level of phytase activity.

DISCUSSION

IN VITRO STUDY

The interaction between Ca and phytate has been extensively studied. Calcium is a dominant divalent metal ion in most diets and has the ability to bind with phytate to form an insoluble complex (Maddaiah et al., 1964b). It is thought that this complex is resistant to

TABLE 13. Effect of dietary calcium, cholecalciferol (D_3), and supplemental phytase on toe ash percentage and phosphorus excretion of broilers fed wheat/canola meal-based diets

nP (%)	Ca (%)	D_3 (IU)	Phytase added (U/kg)	Toe ash (%)	P excretion (g/kg DM)
0.25	0.7	1000	0	8.0^{c}	11.1^{bc}
0.25	0.7	5000	0	9.4^{bc}	10.8^{bc}
0.25	0.7	1000	1000	9.8^{b}	9.6^{d}
0.25	0.7	5000	1000	9.4^{bc}	10.2^{cd}
0.25	0.8	1000	0	9.4^{bc}	11.4^{b}
0.25	0.8	5000	0	9.3^{bc}	11.1^{bc}
0.25	0.8	1000	1000	10.1^{b}	9.8^{d}
0.25	0.8	5000	1000	10.8^{ab}	9.3^{d}
0.45	1.0	1000	0	11.9^{a}	14.9^{a}
SEM				0.5	0.3

[a-d]Means in the same column with different letters differ significantly ($P<.05$)

TABLE 14. Phytase activity (U/kg) of dietary treatments

nP (%)	Ca (%)	D_3 (IU)	Phytase (U/kg)	Analyzed phytase activity (U/kg)	% C.V
0.25	0.7	1000	0	730	5.3
0.25	0.7	5000	0	590	6.1
0.25	0.7	1000	1000	1660	6.2
0.25	0.7	5000	1000	1611	5.4
0.25	0.8	1000	0	661	7.6
0.25	0.8	5000	0	613	3.0
0.25	0.8	1000	1000	1499	3.3
0.25	0.8	5000	1000	1695	5.6
0.45	1.0	1000	0	633	3.0

enzymatic hydrolysis thereby preventing phytate degradation.

The molar ratio of calcium to phytate has been shown to be an integral factor in the formation of calcium phytate complexes. Grynspan and Cheryan (1983) examined the solubility characteristics of calcium phytate complexes over a wide pH range and Ca:PA molar ratios. It was found that at low molar ratios (0.5 to 2.7), very little insoluble complex was formed throughout the pH range, however, as the molar ratio of Ca:PA increased from 4 to12.7, the solubility of calcium and phytate decreased. It was also found that at high Ca:PA molar ratios when pH increased to 5, similar to the pH of the small intestine, there was a dramatic decrease in phytate solubility. At relatively high Ca:PA molar ratios there was greater calcium phytate complex formation and increased insolubility of the complex. In the study presented herein, the addition of Ca or increase in Ca:PA molar ratio from 4 to 12.5 resulted in a decrease in phytate hydrolysis. The decrease in phytate hydrolysis at high Ca levels under our simulated GI tract conditions is very similar to the decrease in phytate solubility presented by Grynspan and Cheryan (1983) at high Ca:PA molar ratios. Metal bound phytate is not readily hydrolyzed by phytase in the pH range of the digestive tract (Reddy et al., 1989). Thus, the presence of insoluble calcium phytate could explain the decrease in phytate hydrolysis in high Ca diets.

The physical attributes of calcium phytate may explain the decreased hydrolysis of phytate at high levels of Ca. The size of particles or aggregates in calcium phytate has been shown to increase as the molar ratio of Ca:PA increased (Campbell et al., 1981). It was found that at a molar ratio of 2:1, there is a population of particles 0.1-0.2 μm in diameter, whereas at a molar ratio of 6:1 these aggregates can grow to 6 μm long. Wise (1983)

predicted that the smaller particle sizes could be associated with more ready dissolution of the complex and consequently a greater susceptibility to hydrolysis by phytase. The larger particles will have a decrease in relative surface area and be more limited to enzymatic attack.

High levels of Ca may also affect the activity of phytase enzyme. Roberts and Yudkin (1961) found that rats fed diets containing high levels of Ca had reduced levels of phytase activity in the intestinal mucosa. This may be attributed to the ability of calcium phytate to bind zinc (Cheryan, 1980). It has been shown that a purified preparation of mucosal phytase is activated by zinc ions (Bitar and Reinhold, 1972). The decreased availability of zinc may reduce phytase activity and consequently phytate hydrolysis. It may also be speculated that at high levels of Ca, Ca^{++} ions invade the active site or attach themselves to the enzyme and alter its configuration, thereby inhibiting phytase activity. The decrease in phytase activity may also be due to the change in pH brought about by adding calcium carbonate into the system. The final step of the *in vitro* procedure involves increasing the pH to 6.0, which simulates the pH of the small intesine. By increasing Ca through the addition of calcium carbonate we may have altered the pH in the system to above pH 6. Phytase enzyme is pH dependent and shows minimal activity near pH 7 (Heinzl, 1996). If the pH approaches this level phytase activity may be very low, consequently, reducing hydrolysis of phytate.

The *in vitro* procedure used in this study closely mimics the conditions of the GI tract of the chicken in terms of pH and temperature. The solubility behaviour of phytate and degree of phytate hydrolysis *in vitro* may be useful for interpretation of the *in vivo* results.

IN VIVO STUDY

It has been shown that dietary Ca and vitamin D_3 affect the utilization of phytate P (Ballam et al., 1985; Edwards, 1993). Most of the recent research on the effect of microbial phytase on phytate P utilization has been conducted with one level of dietary Ca and vitamin D_3 (Simons et al., 1990; Perney et al., 1993). The results of the present study demonstrate that phytase supplementation to low Ca diets improves phytate P utilization as indicated by growth performance, phytate digestibility, toe ash percentage, and P excretion. These findings are in agreement with the previous studies with broiler chickens (Sebastian et al., 1996b) and turkeys (Qian et al., 1996a). Calcium may also have been made more available with the addition of phytase as indicated by improved Ca digestibility in phytase containing diets. Kornegay and Denbow (1996) found that supplemental microbial phytase was effective in improving the bioavailability of Ca in young turkeys fed a low Ca, adequate P corn/SBM based diet. The study demonstrated that the average Ca equivalency estimate of phytase was 0.87 g per 500 units of microbial phytase based on body weight gain, gain:feed, and digestible Ca.

Broiler chickens fed a wheat/canola meal diet with low nP (0.25%) and Ca (0.7%) supported normal growth and feed intake comparable to chicks fed 0.45% nP and 1% Ca. However, toe ash percentage was much lower in birds fed low nP and Ca indicating that the Ca and P requirement for growth is different than the requirement for bone development. It is difficult to explain why birds fed 0.8% Ca responded negatively whereas those fed 0.7% Ca showed a response similar to that of the positive control. The Ca:tP ratios may explain why this discrepancy has occurred. The Ca:tP ratios of diets containing 0.7, 0.8, and 1.0% Ca is 1.06, 1.36, and 1.16:1, respectively, based on analyzed tP and Ca content of diets. The

widest Ca:tP ratio is at 0.8% Ca and may explain why a negative response was attained with these diets. Vandepopuliere et al. (1961) demonstrated that optimum growth performance was attained when chicks were fed a diet at a Ca:tP ratio of 1:1. The increase in Ca:tP ratio which occurs when Ca is increased from 0.7 to 0.8% may produce the negative response, however, this increase is so small it is still difficult to attribute this difference solely to the Ca:tP ratios.

The hydrolysis of phytate and utilization of phytate P has been shown to be influenced by dietary Ca (Ballam et al., 1984; Farkvam et al., 1989). Previous studies with pigs fed a rapeseed diet revealed that high levels of dietary Ca decreases phytate degradation (Sandberg et al., 1993). When calcium intakes were 4.5, 9.9, and 15 g/d the total gastrointestinal degradation of phytate in a rapeseed diet was 97, 77, and 42%, respectively. The results of the present study showed that at 1% Ca phytate digestibility was 43% compared to 53% in low Ca diets. Phytase supplementation further improved phytate digestibility up to 68% in birds fed 0.8% Ca and 5000 IU cholecalciferol. This is in agreement with the findings of Mohammed et al. (1991) who reported that phytate P utilization was increased by 15% when dietary Ca was reduced from 1.0% to 0.5%. The study revealed that the lowering of dietary Ca and elevation of cholecalciferol on low P diets restored body weight gain and tibia toe ash to levels comparable to a control diet. It was also shown that high intakes of cholecalciferol and low intake of Ca increased phytate P digestibility in low P diets. This is in contrast to the results of the present study which found that increasing dietary cholecalciferol to 5000 IU did not improve phytate digestibility. Studies by Edwards (1993) found that supplementation of a corn/SBM diet with the active form of vitamin D_3, 1,25-$(OH)_2D_3$, increased the utilization

of phytate P in broiler chickens. In our study, the extra cholecalciferol added may not have been sufficiently converted to the active form in the liver and kidney, thus, no response was detected. The possible explantation for increased phytate P utilization at high cholecalciferol or 1,25-$(OH)_2D_3$ levels as suggested by Ravindran (1995a) may be due to: 1) increased synthesis or activity of intestinal phytase (Davies et al., 1970); 2) increased Ca absorption rendering phytate more soluble and available for hydrolysis (Wise, 1983); or 3) enhanced transport of P (Wasserman and Taylor, 1973).

In the present study, the addition of phytase to moderately low (0.8%) Ca diets showed the optimum response in growth performance, phytate digestibility, and reduced P excretion. In a similar study conducted with pigs it was found that microbial phytase improved phytate utilization at moderately low levels of dietary Ca than at normally recommended levels (Lei et al., 1994). Qian et al. (1996a) showed that supplemental phytase improved growth performance, toe ash content, and Ca and P retention of turkey poults. These improvements were shown to be negatively influenced by wide dietary Ca:tP ratios. Maximum responses to supplemental phytase was achieved when poults were fed diets with 600 to 900 U of phytase/kg at dietary Ca:tP ratios of 1.1 to 1.4:1. These dietary Ca:tP ratios are similar to those used in the present study. In a study recently conducted with broiler chickens, Sebastian et al. (1996b) found that dietary Ca levels had a significant effect on the response to phytase supplementation with the optimum growth performance and mineral utilization achieved at a low (0.6%) level of dietary Ca.

Phytate has the ability to interact with Ca to form an insoluble calcium phytate complex (Wise, 1983), rendering Ca and P unavailable for intestinal absorption. In the study

described herein , the addition of phytase to diets low in Ca improved Ca digestibility which appeared to be a result of phytate hydrolysis and the release of Ca bound to phytate making it more available for absorption. In the previous *in vitro* study it was shown that at high Ca:PA molar ratios phytate hydrolysis was reduced. It is suggested that in low Ca diets, the Ca-phytate complex is less readily formed in the intestine (Wise, 1983), thus, more phytate is available for hydrolysis. The Ca:PA molar ratios of diets containing 0.7, 0.8, and 1% Ca are 8.75, 10, and 12.5, respectively. The *in vitro* hydrolysis of canola meal phytate with 2000 U/kg phytase somewhat parallels the digestibility of phytate *in vivo*. For example, at a molar ratio of 10 the percentage phytate hydrolysis *in vitro* was 60% (Figure 11) and was comparable to the 61.6% phytate digestibility observed *in vivo* (Table 12).

CHAPTER 5

GENERAL DISCUSSION

There has been relatively little research conducted on the use of supplemental phytase in wheat/canola meal-based diets. In our studies, a wheat/canola meal diet was utilized to examine the effect of phytase supplementation on phytate digestibility, P availability, P excretion, and broiler chicken performance.

An *in vitro* procedure which simulated the GI tract conditions of the chicken was used to examine the effect of phytase on phytate digestibility in canola meal, wheat, wheat/canola meal mixtures, and a wheat/canola meal diet. It was found that the addition of phytase to canola meal resulted in complete dephosphorylation of canola phytate. This is in agreement with research by Zyla and Koreleski (1993) in which complete conversion of rapeseed phytate to lower phosphate esters was accomplished at 40°C and pH 4.5 following phytase addition. In the current study, however, only partial hydrolysis was attained when phytase was added to wheat and a wheat/canola meal diet. It appears that the phytate in wheat may be partially inaccessible or protected by certain layers in the seed thereby limiting phytate hydrolysis by the phytase enzyme. Han and Wilfred (1988) found that soybean phytate was more readily and extensively hydrolyzed than cottonseed phytate. The authors attributed the differences in phytate hydrolysis to the distinct cellular location of phytate in these feedstuffs.

Phytate hydrolysis of a wheat/canola meal diet was found to be significantly lower than that in a wheat/canola meal mixture (Table 6). This indicates that it is not only the partial hydrolysis of wheat phytate which is responsible for the incomplete hydrolysis of wheat/canola meal diets. It appears that there may be a component(s) in the diet which is

affecting enzyme activity and/or substrate availability. In the past there has been much research conducted on the effect of Ca on phytate digestibility (Nahapetian and Young, 1980; Ballam et al., 1984; Sandberg et al., 1993). Calcium has been shown to influence phytate digestibility and phytate P availability through the formation of an insoluble Ca-phytate complex (Wise, 1983). It appears that a soluble substrate may be necessary for phytase to function effectively. Under conditions of high Ca, as in poultry diets, an insoluble Ca-phytate complex which is resistant to enzymatic attack may be formed. This was demonstrated in the *in vitro* study whereby the addition of Ca to canola meal caused a decrease in the rate of phytate hydrolysis (Figure 11). At all levels of phytase supplementation, as Ca:PA molar ratio increased the degree of phytate hydrolysis decreased. It is uncertain if the level of Ca in the diet is the only factor influencing phytate digestibility. Other components such as amino acids, inorganic phosphate, or other minerals may also be affecting enzyme activity and/or substrate solubility.

The use of an *in vitro* procedure which simulates the intestinal conditions is a useful tool to examine enzyme activity and quickly predict the degree of phytate hydrolysis in the presence of digestive enzymes and under different pH conditions. The information gathered from the *in vitro* experimentation can then be reevaluated *in vivo* to determine the efficacy of enzyme supplementation.

The data from the *in vivo* studies indicate that phytase has a positive effect on weight gain and feed intake of broiler chickens fed low nP wheat/canola meal diets, however, no response to phytase supplementation was observed in diets containing 0.35% nP. Therefore, the nP requirement of 0.45% may be an overestimate or may change to 0.35% as the bird

grows older. It appears that 0.35% nP provides sufficient P for growth and bone development of the young chicken, therefore, no response to phytase was detected at this nP level. All diets which contained 0.25% nP responded positively to phytase supplementation. The only exception to this was the second *in vivo* study in which lowering Ca to 0.7% resulted in bird performance equal to that of the positive control. This data was quite surprising and may be explained by the Ca to total P ratio which at the Ca level of 0.7%, approached the optimal 1:1 ratio for broiler chickens. Generally, supplementing 1000 units phytase/kg to 0.25% nP diet increased body weight gain and feed intake of broilers by 8-12%. These results are in agreement with previous findings (Broz et al.,1994; Kornegay et al., 1996). Only minor improvements in feed to gain ratio were noted.

There are few studies in the literature examining phytase and its influence on phytate digestibility. The results of our studies showed that phytate digestibility was significantly improved through phytase supplementation. The addition of phytase increased phytate digestibility by approximately 10 percentage points compared to unsupplemented low nP wheat/canola meal diets. It was also found that intestinal phytase and/or endogenous wheat phytase was able to hydrolyze a good portion of dietary phytate as evidenced by the phytate digestibility figure of approximately 35-50% for a wheat/canola meal diet without enzyme supplementation. In the previous *in vitro* study it was found that 46% of the phytate was hydrolyzed in a wheat/canola meal diet supplemented with 1000 units phytase/kg. The phytate digestibility of a similar diet fed *in vivo* was found to be comparable at 50-65%.

High levels of Ca are known to affect phytate digestibility in poultry (Ballam et al., 1984; Scheidler and Sell, 1987). In our second *in vivo* study it was difficult to confirm the

negative effect of Ca on phytate digestibility and phytate P utilization since a high or normal Ca level was not used with a low nP diet. Another set of dietary treatments should have been utilized which contained 0.25% nP and 1% Ca. This would have allowed a direct comparison between normal (1%) and low (0.7 or 0.8%) Ca levels.

The improvement in phytate digestibility resulted in the liberation of phosphate groups which were subsequently absorbed as indicated by increased body weight gain and toe ash percentage. Birds fed a low nP (0.25%) diet had a toe ash percentage much lower than the positive control. In the first *in vivo* study, the addition of phytase to low nP diets increased toe ash percentage, however, it did not reach the level of the positive control. In the study where Ca was reduced to 0.8%, the addition of 1000 units phytase/kg improved toe ash percentage to a level comparable to that of positive control. Although birds fed 0.25% nP and 0.7% Ca grew as efficiently as those fed the positive control, the toe ash percentage was significantly lower regardless of phytase supplementation.

Phosphorus excretion (g/kg DM) was shown to be reduced through both decreased nP and level of phytase supplementation. The magnitude of response to phytase supplementation was greater at 0.25% nP than at 0.35% nP. In comparison with a positive control (0.45% nP), supplementation of a 0.25% nP diet with 1000 units phytase/kg reduced P excretion by 35%. The results indicate that microbial phytase provides a means of reducing P pollution. In a province such as Manitoba which raises 20 million broilers per year the use of microbial phytase may greatly aid in reducing the phosphate burden on the environment. It may be estimated that approximately 324 tonnes of P are annually produced as a waste product by broilers in Manitoba. A 35% reduction would mean 113 tonnes less P being

excreted by broilers annually. Other practical methods to alleviate the phosphate burden include avoiding over-use of inorganic phosphates, formulating diets more precisely, using phase feeding and split-sex feeding, and selecting feedstuffs in which P is highly available. The use of microbial phytase in poultry diets will depend on a number of factors including the environmental need to reduce the excretion of P and other minerals, costs of inorganic phosphate, and phytase.

In comparison to corn/SBM diet, wheat/canola meal diet contains nearly twice the amount of phytate P (0.22 vs. 0.46%). It is questionable as to whether higher amounts of phytase are required to be supplemented into a wheat/canola meal diet (due to the higher phytate content) to achieve a similar response as that observed for a corn/SBM diet. Ravindran et al. (1995b) found that in corn/SBM diets containing 0.27% nP and supplemented with different levels of phytase, body weight gains improved up to 800 units of phytase/kg of diet at which point a plateau was reached. In the current experiments, 1000 units phytase/kg of diet produced responses equal to that of the positive control, whereas, 500 units phytase/kg produced a somewhat lower response. Further studies with wheat/canola meal diets may be needed to precisely establish the level of supplemental phytase.

CHAPTER 6

SUMMARY AND CONCLUSION

The present research has demonstrated the positive effects of phytase supplementation on phytate digestibility, P availability, P excretion, and broiler chicken performance. *In vitro* experiments indicated improved phytate hydrolysis with phytase addition to canola meal, wheat, and wheat/canola meal diets. This was substantiated in the *in vivo* experimentation and reflected in the growth performance and toe ash percentage of birds fed enzyme supplemented diets compared to their nonsupplemented counterparts. The present data suggest that low nP wheat/canola meal diets supplemented with 1000 units phytase/kg is adequate to support chick growth. Calcium was shown to affect phytate hydrolysis in both *in vitro* and *in vivo* experiments. Birds fed low Ca and nP diets supplemented with phytase had growth rates comparable to those fed a positive control.

The following conclusions can be drawn from the data collected:

(1) The degree of phytate dephosphorylation differs among canola meal and wheat. Under simulated conditions of the GI tract, phytase was found to completely hydrolyze the phytate in canola meal, whereas, only partial hydrolysis (ie.,46%) was achieved in wheat.

(2) The particle size or degree of grinding of wheat appears to influence extent of phytate hydrolysis *in vitro*, in which smaller particle size results in greater hydrolysis of phytate.

(3) The addition of phytase to wheat/canola meal diet resulted in even lower degree of phytate hydrolysis *in vitro* which may be a consequence of negative effects of certain dietary components on enzyme activity.

(4) The addition of Ca to canola meal affected the degree of phytate hydrolysis *in vitro* indicating the negative effect of this mineral on either enzyme activity or substrate solubility.

(5) Magnitude of response to phytase supplementation is dependent on the level of nP with the greatest response occurring at low nP levels.

(6) Supplementing phytase to low nP wheat/canola meal diets results in improved broiler chicken performance as indicated by weight gain and feed intake.

(7) Phytate P availability and phytate and Ca digestibility of wheat/canola meal diets are increased through phytase supplementation.

(8) P excretion can be reduced by up to 37% through phytase supplementation of wheat/canola meal diets.

(9) Lower levels of Ca may be used in phytase supplemented low nP wheat/canola meal diets.

Further research is suggested to:

(1) examine the effect of phytase supplementation and reduced Ca level on broiler chicken performance in a six week production trial.

(2) investigate the effect of microbial phytase on Ca, trace mineral, and amino acid utilization.

(3) establish the optimum inclusion level of phytase for wheat/canola meal diets.

(4) explore the potential for development of an enzyme "cocktail" with activities facilitating the hydrolysis of phytate by phytase enzyme.

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APPENDIX TABLES

TABLE A1. Main effects of calcium, cholecalciferol, and supplemental phytase on feed intake, body weight gain, and feed:gain ratios of broilers fed wheat/canola meal diets

Source of variation	Feed intake (g/bird)	Weight gain (g/bird)	Feed:Gain
Calcium (Ca)	.0001	.0014	NS
Phytase (P)	.0030	.0003	.0119
Cholecalciferol (D)	NS	NS	NS
Ca*P	.0146	.0206	NS
Ca*D	NS	NS	NS
P*D	.0331	NS	NS
Ca*P*D	NS	NS	NS

TABLE A2. Main effects of calcium, cholecalciferol, and supplemental phytase on phytate and calcium digestibility of broilers fed wheat/canola meal-based diets

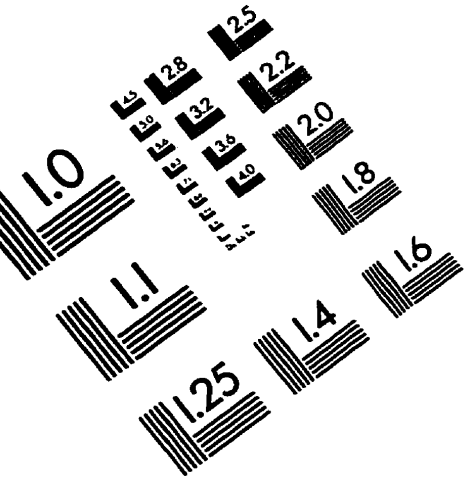
Source of variation	Phytate dig.[1] (%)	Phytate dig.[2] (%)	Calcium dig.[2] (%)
Calcium (Ca)	.0088	.3913	NS
Phytase (P)	.0001	.0001	.0001
Cholecalciferol (D)	NS	.0240	NS
Ca*P	NS	NS	NS
Ca*D	NS	NS	NS
P*D	NS	NS	NS
Ca*P*D	NS	.0401	NS

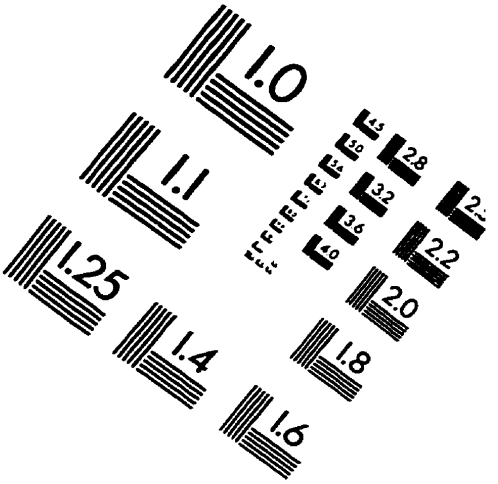
[1]as determined at the terminal ileum
[2]as determined in the excreta

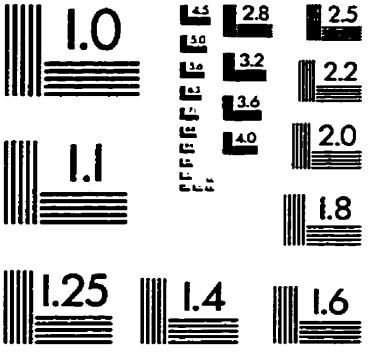
TABLE A3. Main effects of calcium, cholecalciferol, and supplemental phytase on toe ash percentage and phosphorus excretion of broilers fed wheat/canola meal-based diets

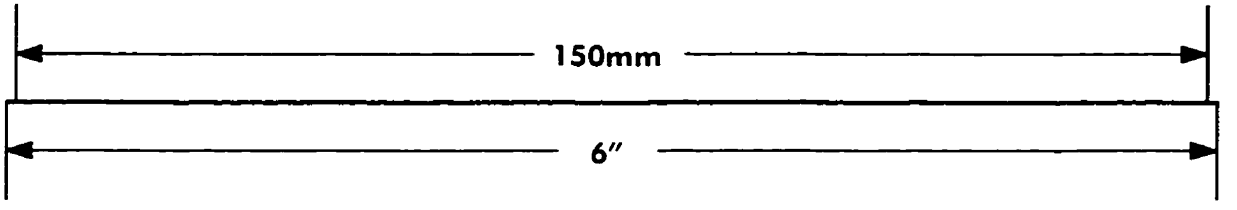
Source of variation	Toe Ash (%)	P excretion (g/kgDM)
Calcium (Ca)	NS	NS
Phytase (P)	.0165	.0001
Cholecalciferol (D)	NS	NS
Ca*P	NS	NS
Ca*D	NS	NS
P*D	NS	NS
Ca*P*D	NS	NS

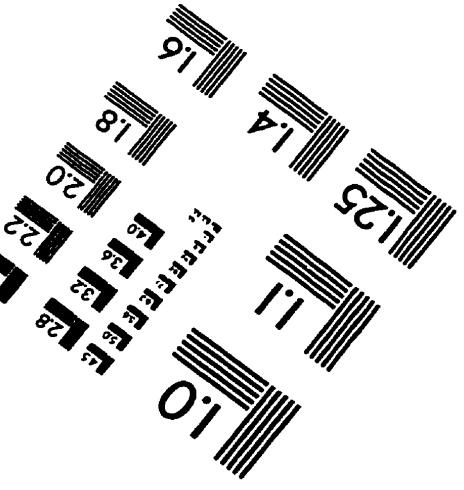
IMAGE EVALUATION
TEST TARGET (QA-3)

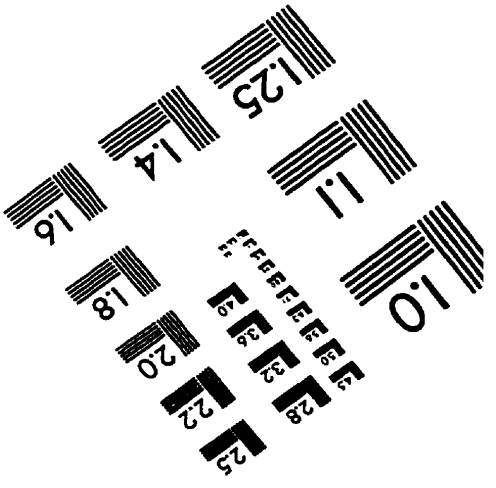












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